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                   alerts (SDIs) affected
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                   alerts (SDIs) affected
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L1             0 CLOSTRIDIUM TOXIN COMPLEX

=> s clostridium toxin fusion protein
L2             0 CLOSTRIDIUM TOXIN FUSION PROTEIN

=> s clostridium toxin
L3          1905 CLOSTRIDIUM TOXIN

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L4            15 L3 AND TARGETING

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L5   ANSWER 1 OF 14  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
2004181008  EMBASE  pH-sensitive toxins: Interactions with membrane bilayers
     and application to drug delivery. Cabiaux V.. V. Cabiaux, Universite Libre
     de Bruxelles, Struct. Funct. of Biol. Membranes, 2 Boulevard du Triomphe,
     1050 Brussels, Belgium. vcabiaux@ulb.ac.be. Advanced Drug Delivery Reviews
     56/7 (987-997)  23 Apr 2004.
     Refs: 46.
     ISSN: 0169-409X. CODEN: ADDREP.
     Publisher Ident.: S 0169-409X(03)00281-3. Pub. Country: Netherlands.
     Language: English. Summary Language: English.
AB   pH-sensitive toxins are secreted by bacteria and reach the cytosol of
     eukaryotic target cells by complex mechanisms involving receptor binding,
     membrane interaction and translocation across a cell lipid membrane.
     Membrane interaction and ability to reach the cytoplasm have been used
     respectively to present proteins at the cell surface and to transport
     foreign peptides or DNA into the cytoplasm. The first approach is used in
     anticancer vaccination and the second in inducing a major
     histocompatibility (MHC) class I presentation of exogenous peptides or
     proteins. A brief overview of the use of toxins themselves for
     targeting cancer cells is also presented. Altogether, the data
     suggest that pH sensitive toxins have a huge potential for surface
     presentation or cytosol transport of biomacromolecules and that many ways
     could still be explored to develop new strategies in vaccination or
     therapeutic methods. .COPYRGT. 2004 Elsevier B.V. All rights reserved.

L5   ANSWER 2 OF 14  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
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on STN
2005002704 EMBASE Bacteria and bacterial toxins as therapeutic agents for solid tumors. Michl P.; Gress T.M.. T.M. Gress, Abt. Innere Medizin I, Universitatsklinikum Ulm, Robert-Koch-Str. 8, 89081 Ulm, Germany. thomas.gress@medizin.uni-ulm.de. Current Cancer Drug Targets 4/8 (689-702) 2004.
Refs: 116.
ISSN: 1568-0096. CODEN: CCDTB. Pub. Country: Netherlands. Language: English. Summary Language: English.
AB Patients with advanced solid tumors frequently relapse and succumb to their metastatic disease after developing resistance to conventional treatment modalities such as chemotherapy and radiotherapy. In these patients, novel strategies of **targeting** widespread tumors are urgently needed. The increasing knowledge of the underlying pathogenetic mechanisms has led to the identification of numerous molecules that are overexpressed in various tumors and accumulate at the cell surface. The use of genetically modified bacteria and their toxins **targeting** these surface molecules has emerged as a promising new treatment strategy in refractory cancers. This review focuses on bacterial toxins such as Diphtheria toxin (DT), Pseudomonas exotoxin A (PE) and Clostridium perfringens enterotoxin (CPE). In addition, the use of anaerobic bacteria such as Clostridium, Salmonella and Bifidobacterium spp. as drug-delivery systems **targeting** hypoxic tumor areas will be discussed as a new therapeutic modality of advanced solid tumors.

L5 ANSWER 3 OF 14 EMBASE
on STN
2004278661 EMBASE Large clostridial cytotoxins: Cellular biology of Rho/Ras-glucosylating toxins. Schirmer J.; Aktories K.. K. Aktories, Inst. F. Experimentelle Klin. P., Albert-Ludwigs-Univ. Freiburg, Otto-Krayer-Haus, Albertstrasse 25, D-79104 Freiburg, Germany. Klaus.Aktories@pharmakol.uni-freiburg.de. Biochimica et Biophysica Acta - General Subjects 1673/1-2 (66-74) 6 Jul 2004.
Refs: 72.
ISSN: 0304-4165. CODEN: BBGSB3.
Publisher Ident.: S 0304-4165(04)00067-4. Pub. Country: Netherlands. Language: English. Summary Language: English.
AB Mono-O-glycosylation of eukaryotic target proteins is the molecular mechanism of bacterial protein toxins of the family of large clostridial cytotoxins. This toxin family encompasses several high molecular mass proteins (>250 kDa) of various Clostridia species that are implicated in severe human diseases. Toxin A and toxin B from Clostridium difficile are the causative agents of pseudomembranous colitis and antibiotic-associated diarrhea. Lethal toxin and hemorrhagic toxin from Clostridium sordellii as well as α-toxin from Clostridium novyi are involved in the gas gangrene syndrome. The common mode of action of large clostridial cytotoxins is elicited by specific glycosylation of small GTP-binding proteins in the cytosol of target cells using activated nucleotide sugars as cosubstrates. Specific modification at a single threonine residue in the small GTPases renders these important key players of various signaling pathways inactive. This minireview intends to give an overview on structure-function analysis and mode of action of the large clostridial cytotoxins, as well as on the resulting functional consequences of glycosylation of target proteins.

L5 ANSWER 4 OF 14 EMBASE
on STN
2004316705 EMBASE Retargeted clostridial endopeptidases: Inhibition of nociceptive neurotransmitter release in vitro, and antinociceptive activity in vivo models of pain. Chaddock J.A.; Purkiss J.R.; Alexander F.C.G.; Doward S.; Fooks S.J.; Friis L.M.; Hall Y.H.J.; Kirby E.R.; Leeds N.; Moulsdale H.J.; Dickenson A.; Green G.M.; Rahman W.; Suzuki R.; Duggan M.J.; Quinn C.P.; Shone C.C.; Foster K.A.. Dr. J.A. Chaddock, Health Protection Agency, Porton Down, Salisbury, Wiltshire SP4 0JG, United

Kingdom. john.chaddock@hpa.org.uk. Movement Disorders 19/SUPPL. 8 (S42-S47) 2004.
Refs: 22.
ISSN: 0885-3185. CODEN: MOVDEA. Pub. Country: United States. Language: English. Summary Language: English.

AB Clostridial neurotoxins potently and specifically inhibit neurotransmitter release in defined cell types. Previously reported data have demonstrated that the catalytically active LH(N) endopeptidase fragment of botulinum. neurotoxin type A (termed LH(N)/A) can be retargeted to a range of cell types in vitro to lead to inhibition of secretion of a range of transmitters. Here, we report the synthesis of endopeptidase conjugates with in vitro selectivity for nociceptive afferents compared to spinal neurons. Chemical conjugates prepared between Erythrina cristagalli lectin and LH(N)/A are assessed in vitro and in in vivo models of pain. Chemical conjugates prepared between E. cristagalli lectin and either natively sourced LH(N)/A, or recombinant LH(N)/A purified from Escherichia coli are assessed, and equivalence of the recombinant material is demonstrated. The duration of action of inhibition of neurotransmitter release by the conjugate in vitro is also assessed and is comparable to that observed with Clostridium botulinum neurotoxin. Selectivity of **targeting** and therapeutic potential have been confirmed by in vivo electrophysiology studies. Furthermore, the analgesic properties of the conjugate have been assessed in in vivo models of pain and extended duration effects observed. These data provide proof of principle for the concept of retargeted clostridial endopeptidases as novel analgesics. .COPYRGT. 2004 Movement Disorder Society.

L5 ANSWER 5 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN
2003:696462 Document No. 139:207733 Construction of recombinant single-chain toxins for use in vaccines and toxin assays. Shone, Clifford Charles; Quinn, Conrad Padraig; Foster, Keith Alan; Chaddock, John; Marks, Philip; Sutton, J. Mark; Stancombe, Patrick; Wayne, Jonathan (Microbiological Research Authority, UK; Speywood Laboratory Limited). U.S. Pat. Appl. Publ. US 2003166238 A1 20030904, 37 pp., Cont.-in-part of U.S. Ser. No. 255,829. (English). CODEN: USXXCO. APPLICATION: US 2002-241596 20020912. PRIORITY: GB 1996-17671 19960823; GB 1996-25996 19961213; US 1996-782893 19961227; WO 1997-GB2273 19970822; US 1999-255829 19990223; US 1999-242689 19990223.

AB A single-chain polypeptide is provided which comprises first and second domains. The first domain enables the polypeptide to cleave one or more vesicle or plasma-membrane associated proteins essential to exocytosis, and the second domain enables the polypeptide to be translocated into a target cell or increases the solubility of the polypeptide, or both. The polypeptide thus combines useful properties of a clostridial toxin, such as a botulinum or tetanus toxin, without the toxicity associated with the natural mol. The polypeptide can also contain a third domain that targets it to a specific cell, rendering the polypeptide useful in inhibition of exocytosis in target cells. Fusion proteins comprising the polypeptide, nucleic acids encoding the polypeptide and methods of making the polypeptide are also provided. Controlled activation of the polypeptide is possible and the polypeptide can be incorporated into vaccines and toxin assays.

L5 ANSWER 6 OF 14 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2003370100 EMBASE The journey of tetanus and botulinum neurotoxins in neurons. Lalli G.; Bohnert S.; Deinhardt K.; Verastegui C.; Schiavo G.. G. Lalli, MRC Lab. for Molecular Cell Biology, University College London, Gower Street, London, WC1E 6BT, United Kingdom. giampietro.schiavo@cancer.org.uk. Trends in Microbiology 11/9 (431-437) 1 Sep 2003.
Refs: 65.
ISSN: 0966-842X. CODEN: TRMIEA. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Anaerobic bacteria of the genus Clostridia are a major threat to human and

animal health, being responsible for pathologies ranging from food poisoning to gas gangrene. In each of these, the production of sophisticated exotoxins is the main cause of disease. The most powerful clostridial toxins are tetanus and botulinum neurotoxins, the causative agents of tetanus and botulism. They are structurally organized into three domains endowed with distinct functions: high affinity binding to neurons, membrane translocation and specific cleavage of proteins controlling neuroexocytosis. Recent discoveries regarding the mechanism of membrane recruitment and sorting of these neurotoxins within neurons make them ideal tools to uncover essential aspects of neuronal physiology in health and disease.

L5 ANSWER 7 OF 14 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 1

2003159782 EMBASE The sensitivity of catecholamine release to botulinum toxin C1 and E suggests selective **targeting** of vesicles set into the readily releasable pool. Stigliani S.; Raiteri L.; Fassio A.; Bonanno G.. Dr. G. Bonanno, Dipto. di Medicina Sperimentale, Sezione di Farmacol. e Tossicologia, Viale Cembrano 4, 16148 Genova, Italy. bonnanno@pharmatox.unige.it. Journal of Neurochemistry 85/2 (409-421) 2003.
Refs: 57.
ISSN: 0022-3042. CODEN: JONRA. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB The impact of syntaxin and SNAP-25 cleavage on [(3)H]noradrenaline ([(3)H]NA) and [(3)H]dopamine ([(3)H]DA) exocytotic release evoked by different stimuli was studied in superfused rat synaptosomes. The external Ca(2+)-dependent K(+)-induced [(3)H]catecholamine overflows were almost totally abolished by botulinum toxin C1 (BoNT/C1), which hydrolyses syntaxin and SNAP-25, or by botulinum toxin E (BoNT/E), selective for SNAP-25. BoNT/C1 cleaved 25% of total syntaxin and 40% of SNAP-25; BoNT/E cleaved 40% of SNAP-25 but left syntaxin intact. The GABA uptake-induced releases of [(3)H]NA and [(3)H]DA were differentially affected: both toxins blocked the former, dependent on external Ca(2+), but not the latter, internal Ca(2+)-dependent. BoNT/C1 or BoNT/E only slightly reduced the ionomycin-evoked [(3)H]catecholamine release. More precisely, [(3)H]NA exocytosis induced by ionomycin was sensitive to toxins in the early phase of release but not later. The Ca(2+)-independent [(3)H]NA exocytosis evoked by hypertonic sucrose, thought to release from the readily releasable pool (RRP) of vesicles, was significantly reduced by BoNT/C1. Pre-treating synaptosomes with phorbol-12-myristate-13-acetate, to increase the RRP, enhanced the sensitivity to BoNT/C1 of [(3)H]NA release elicited by sucrose or ionomycin. Accordingly, cleavage of syntaxin was augmented by the phorbol-ester. To conclude, our results suggest that clostridial toxins selectively target exocytosis involving vesicles set into the RRP.

L5 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN

2002:968399 Document No. 138:215989 Detection of toxigenic clostridia. Popoff, Michel R. (Unite des Toxines Bacteriennes, Institut Pasteur, CNR Anaerobies, Institut Pasteur, Paris, Fr.). Methods in Molecular Biology (Totowa, NJ, United States), 216(PCR Detection of Microbial Pathogens), 137-152 (English) 2003. CODEN: MMBIED. ISSN: 1064-3745. Publisher: Humana Press Inc..

AB The main difficulty of identifying Clostridium spp. using classical methods is that most of the pathogenic Clostridium spp. are strictly anaerobic bacteria, which hampers their isolation from biol. samples and identification by culture. Since most of the clostridial toxin genes are well characterized, this permits the use of mol. methods for detection and identification of toxigenic Clostridium species from biol. and food samples. This chapter describes the identification of toxigenic Clostridium species by polymerase chain reaction (PCR) methods **targeting** toxin genes.

L5 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN

2002:927279 Document No. 138:29103 Pharmaceutical use for secreted bacterial effector proteins. Sutton, John Mark; Shone, Clifford Charles (Microbiological Research Authority, UK). PCT Int. Appl. WO 2002096467 A2 20021205, 130 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-GB2384 20020521. PRIORITY: GB 2001-12687 20010524.

AB A polypeptide conjugate contains a bacterial injectable effector protein, secreted by a modified pilus or "needle-like" structure comprising a type III or type IV secretion apparatus, and a carrier that targets the conjugate to a target cell. The effector protein is used for a variety of purposes including treatment of neurodegenerative disease, intracellular infection and diseases associated with defects of secretion.

L5 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN

2002:256064 Document No. 136:293508 New immunogenic complex comprising glycoside, lipid, iscom matrix, antigen and enzyme. Lycke, Nils; Dalsgaard, Kristian; McMowat, Allan; Loewenadler, Bjoern; Kaastrup, Peter (Isconova Ab, Swed.). PCT Int. Appl. WO 2002026255 A1 20020404, 64 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-SE2117 20011001. PRIORITY: SE 2000-3538 20000929.

AB The invention relates to an immunogenic complex comprising at least one glycoside and at least one lipid, integrated into an iscom complex or matrix, and at least one antigen which antigen is integrated into the iscom complex or coupled on to or mixed with the iscom complex or iscom matrix complex, characterized in that it also comprises at least one enzyme. The enzyme confers ADP-ribosylating activity and is derived from cholera toxin, Escherichia coli heat labile enterotoxin, pertussis toxin, **Clostridia toxin**, Shigella toxin or pseudomonas toxin.
It also relates to such a complex comprising at least one peptide which specifically binds to a receptor expressed on a cell capable of antigen presentation, which cell expresses MHC Class I or Class II and to compns. comprising the complexes. The antigen presenting cell is selected from lymphocytes, macrophages, dendritic cells, Langerhans cells and epithelial cells. The immunogenic complex further comprises other immunomodulatory compds or **targeting** agents for pharmaceutical and veterinary use.

L5 ANSWER 11 OF 14 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2002367087 EMBASE Clostridial neurotoxins. Verastegui C.; Lalli G.; Bohnert S.; Meunier F.A.; Schiavo G.. C. Verastegui, Molec. NeuroPathobiology Laboratory, Cancer Research UK, London Research Institute, 44 Lincoln's Inn Fields, WC2A 3PX London, United Kingdom. Journal of Toxicology - Toxin Reviews 21/3 (203-227) 2002.
Refs: 129.
ISSN: 0731-3837. CODEN: JTTRD. Pub. Country: United States. Language: English. Summary Language: English.

AB Tetanus (TeNT) and botulinum (BoNTs) neurotoxins are powerful toxins endowed with a specific zinc-endopeptidase activity. Targets of these neurotoxins have been identified as synaptic members of the SNARE proteins, which are involved in the exocytosis of neurotransmitters at the synapse. Despite this identical intracellular mechanism of action, TeNT

and BoNTs target different neurons in vivo. After binding at the neuromuscular junction, BoNTs block neurotransmitter release at this site, whereas TeNT is retrogradely transported through motor neurons and inhibits exocytosis in inhibitory interneurons. Recently, several studies have reported the structure of these neurotoxins and clarified important aspects of the intoxication process. However, important questions on the mechanism responsible for the binding specificity and for the **targeting** of TeNT and BoNTs remain to be addressed. Once elucidated, this novel information would enables us to use CNTs more efficiently as therapeutic tools in neuronal disorders.

L5 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN
2000:381824 Document No. 133:22430 Conjugate for enrichment in neural cells. Loehr, Achim; Schwab, Manfred (Deutsches Krebsforschungszentrum Stiftung des Oeffentlichen Rechts, Germany). Ger. Offen. DE 19856052 A1 20000608, 12 pp. (German). CODEN: GWXXBX. APPLICATION: DE 1998-19856052 19981204.
AB An active substance is introduced specifically into neurons by conjugating it with the heavy chain of a **Clostridium toxin** in the absence of the toxic light chain. The heavy chain and its fragments, modifications, and fusion proteins mediate neuron-specific binding, uptake, and release of the conjugate into the cytoplasm, but are nontoxic. The active agent is coupled to an SH group on the heavy chain through a linker via an SS group; alternatively, if the active agent is a nucleic acid, it is bound via H bonds to an oligonucleotide coupled to the heavy chain, or via ionic bonds to a peptide or protein recombinantly fused to the heavy chain. Use of the heavy chain is facilitated by modifying it to mimic the structure of the native toxin; this is done by substituting other, nontoxic proteins for the light chain and binding them to the heavy chain via SS groups. The conjugates can be used for diagnosis and/or therapy of neuronal diseases, e.g. neuroblastoma. Thus, the gene for tetanus toxin heavy chain was cloned, fused with the gene for HMG-1 (high-mobility-group) protein (which nonspecifically binds DNA), and expressed in Escherichia coli.

L5 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN
1997:776762 Document No. 127:355944 Fusion proteins of immunopotentiating activity and fusions of bacterial toxins with specific cell receptors. Lowenadler, Bjorn; Lycke, Nils (Lowenadler, Bjorn, Swed.; Lycke, Nils). Can. Pat. Appl. CA 2168914 AA 19970807, 28 pp. (English). CODEN: CPXXEB. APPLICATION: CA 1996-2168914 19960206.
AB The claims include a DNA-sequence comprising a first sequence coding for a native or mutant subunit of a bacterial toxin that confers enzymic ADP-ribosylating activity, and a second sequence coding for a peptide such that the resulting fusion protein is in possession of water solubility and capability of **targeting** the fusion protein to a specific cell receptor different from receptors binding to the native toxin, thereby mediating intracellular uptake of at least said subunit. Also claimed are fusion proteins coded for by such DNA-sequence; compns. for use in improving immune functions; and recombinant expression vectors and transformed bacterial cells containing such DNA-sequence. The bacterial toxins may include cholera toxin, Escherichia coli heat-labile enterotoxin, and toxins of Pertussis, Clostridium, Shigella, and Pseudomonas. The cell receptor may be for lymphocytes and monocytes, for Ig or Fc, or for antigen presentation.

L5 ANSWER 14 OF 14 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
97087126 EMBASE Document No.: 1997087126. Bacterial toxins that target rho proteins. Aktories K.. Dr. K. Aktories, Inst. fur Pharmakologie/Toxikologie, Albert-Ludwigs-Univ. Freiburg i.Br., Hermann-Herder-Str. 5, D-79104 Freiburg i.Br., Germany. aktories@ruf.uni-freiburg.de. Journal of Clinical Investigation 99/5 (827-829) 1997.
Refs: 10.
ISSN: 0021-9738. CODEN: JCINAO. Pub. Country: United States. Language:

English.

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L2 0 S CLOSTRIDIUM TOXIN FUSION PROTEIN
L3 1905 S CLOSTRIDIUM TOXIN
L4 15 S L3 AND TARGETING
L5 14 DUP REMOVE L4 (1 DUPLICATE REMOVED)

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L6 0 L3 AND "NON-NEURONAL CELL"

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PROCESSING COMPLETED FOR L7
L8 25 DUP REMOVE L7 (1 DUPLICATE REMOVED)

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L8 ANSWER 1 OF 25 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
2005002704 EMBASE Bacteria and bacterial toxins as therapeutic agents for solid tumors. Michl P.; Gress T.M.. T.M. Gress, Abt. Innere Medizin I, Universitatsklinikum Ulm, Robert-Koch-Str. 8, 89081 Ulm, Germany. thomas.gress@medizin.uni-ulm.de. Current Cancer Drug Targets 4/8 (689-702) 2004.
Refs: 116.
ISSN: 1568-0096. CODEN: CCDTB. Pub. Country: Netherlands. Language: English. Summary Language: English.
AB Patients with advanced solid tumors frequently relapse and succumb to their metastatic disease after developing resistance to conventional treatment modalities such as chemotherapy and radiotherapy. In these patients, novel strategies of targeting widespread tumors are urgently needed. The increasing knowledge of the underlying pathogenetic mechanisms has led to the identification of numerous molecules that are overexpressed in various tumors and accumulate at the cell surface. The use of genetically modified bacteria and their toxins targeting these surface molecules has emerged as a promising new treatment strategy in refractory cancers. This review focuses on bacterial toxins such as Diphtheria toxin (DT), Pseudomonas exotoxin A (PE) and Clostridium perfringens enterotoxin (CPE). In addition, the use of anaerobic bacteria such as Clostridium, Salmonella and Bifidobacterium spp. as drug-delivery systems targeting hypoxic tumor areas will be discussed as a new therapeutic modality of advanced solid tumors.

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on STN
2004316705 EMBASE Retargeted clostridial endopeptidases: Inhibition of nociceptive neurotransmitter release in vitro, and antinociceptive activity in vivo models of pain. Chaddock J.A.; Purkiss J.R.; Alexander F.C.G.; Doward S.; Fooks S.J.; Friis L.M.; Hall Y.H.J.; Kirby E.R.; Leeds N.; Moulsdale H.J.; Dickenson A.; Green G.M.; Rahman W.; Suzuki R.; Duggan M.J.; Quinn C.P.; Shone C.C.; Foster K.A.. Dr. J.A. Chaddock, Health Protection Agency, Porton Down, Salisbury, Wiltshire SP4 0JG, United Kingdom. john.chaddock@hpa.org.uk. Movement Disorders 19/SUPPL. 8 (S42-S47) 2004.
Refs: 22.

ISSN: 0885-3185. CODEN: MOVDEA. Pub. Country: United States. Language: English. Summary Language: English.

AB Clostridial neurotoxins potently and specifically inhibit neurotransmitter release in defined cell types. Previously reported data have demonstrated that the catalytically active LH(N) endopeptidase fragment of botulinum. neurotoxin type A (termed LH(N)/A) can be retargeted to a range of cell types in vitro to lead to inhibition of secretion of a range of transmitters. Here, we report the synthesis of endopeptidase **conjugates** with in vitro selectivity for nociceptive afferents compared to spinal neurons. Chemical **conjugates** prepared between Erythrina cristagalli lectin and LH(N)/A are assessed in vitro and in in vivo models of pain. Chemical **conjugates** prepared between E. cristagalli lectin and either natively sourced LH(N)/A, or recombinant LH(N)/A purified from Escherichia coli are assessed, and equivalence of the recombinant material is demonstrated. The duration of action of inhibition of neurotransmitter release by the **conjugate** in vitro is also assessed and is comparable to that observed with Clostridium botulinum neurotoxin. Selectivity of targeting and therapeutic potential have been confirmed by in vivo electrophysiology studies. Furthermore, the analgesic properties of the **conjugate** have been assessed in in vivo models of pain and extended duration effects observed. These data provide proof of principle for the concept of retargeted clostridial endopeptidases as novel analgesics. .COPYRGT. 2004 Movement Disorder Society.

L8 ANSWER 3 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN

2002:927279 Document No. 138:29103 Pharmaceutical use for secreted bacterial effector proteins. Sutton, John Mark; Shone, Clifford Charles (Microbiological Research Authority, UK). PCT Int. Appl. WO 2002096467 A2 20021205, 130 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-GB2384 20020521. PRIORITY: GB 2001-12687 20010524.

AB A polypeptide **conjugate** contains a bacterial injectable effector protein, secreted by a modified pilus or "needle-like" structure comprising a type III or type IV secretion apparatus, and a carrier that targets the **conjugate** to a target cell. The effector protein is used for a variety of purposes including treatment of neurodegenerative disease, intracellular infection and diseases associated with defects of secretion.

L8 ANSWER 4 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN

2002:256064 Document No. 136:293508 New immunogenic complex comprising glycoside, lipid, iscom matrix, antigen and enzyme. Lycke, Nils; Dalsgaard, Kristian; McMowat, Allan; Loewenadler, Bjoern; Kaastrup, Peter (Isconova Ab, Swed.). PCT Int. Appl. WO 2002026255 A1 20020404, 64 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-SE2117 20011001. PRIORITY: SE 2000-3538 20000929.

AB The invention relates to an immunogenic complex comprising at least one glycoside and at least one lipid, integrated into an iscom complex or matrix, and at least one antigen which antigen is integrated into the iscom complex or coupled on to or mixed with the iscom complex or iscom matrix complex, characterized in that it also comprises at least one

enzyme. The enzyme confers ADP-ribosylating activity and is derived from cholera toxin, Escherichia coli heat labile enterotoxin, pertussis toxin, **Clostridia toxin**, Shigella toxin or pseudomonas toxin.
It also relates to such a complex comprising at least one peptide which specifically binds to a receptor expressed on a cell capable of antigen presentation, which cell expresses MHC Class I or Class II and to compns. comprising the complexes. The antigen presenting cell is selected from lymphocytes, macrophages, dendritic cells, Langerhans cells and epithelial cells. The immunogenic complex further comprises other immunomodulatory compds or targeting agents for pharmaceutical and veterinary use.

L8 ANSWER 5 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN
2002:213816 Document No. 136:241677 Treatment of C. difficile toxin B associated conditions. Heerze, Louis D.; Armstrong, Glen D. (Synsorb Biotech Inc., Can.). U.S. US 6358930 B1 20020319, 14 pp., Cont.-in-part of U.S. 6,013,635. (English). CODEN: USXXAM. APPLICATION: US 1999-433944 19991104. PRIORITY: US 1998-85032 19980528.
AB This invention relates to prevention and/or treatment of antibiotic associated diarrhea, including Clostridium difficile associated diarrhea (CDAD), pseudomembranous colitis (PMC) and other conditions associated with C. difficile infection, using oligosaccharide compns. which bind C. difficile toxin B. More specifically, the invention concerns neutralization of C. difficile toxin B associated with such conditions. Examples are provided on neutralization of C. difficile toxins A and B by SYNSORBs and on effect of preincubation of toxin B with SYNSORBs on transepithelial resistance in human colonic tissue.

L8 ANSWER 6 OF 25 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2001162717 EMBASE Detection of clostridium botulinum neurotoxin type A using immuno-PCR. Wu H.C.; Huang Y.L.; Lai S.C.; Huang Y.Y.; Shaio M.F.. Dr. H.C. Wu, Institute of Preventive Medicine, National Defense Medical Center, PO Box 90048-700, San-Hsia, Taiwan,Province of China. hancw@pchome.com.tw. Letters in Applied Microbiology 32/5 (321-325) 2001.
Refs: 16.
ISSN: 0266-8254. CODEN: LAMIE7. Pub. Country: United Kingdom. Language: English. Summary Language: English.
AB Aims: An immuno-polymerase chain reaction (immuno-PCR) has been developed for the sensitive detection of antigens, which greatly extends the detection limits of immunoassays. In the current study, the method was applied to the detection of Clostridium botulinum neurotoxin type A (BTx-A). Methods and Results: Anti-BTx-A antibody-DNA **conjugates** were synthesized using a heterobifunctional cross-linker reagent to covalently link the reporter DNA and the antibodies. The antibody-DNA **conjugates** with antigens were amplified by PCR, and dose-dependent relationships for each analyte were demonstrated. Detection limits of immuno-PCR for BTx-A (3.33 x 10(-17) mol) exceeded the conventional enzyme-linked immunosorbent assay (3.33 x 10(-14) mol) by a 1000-fold enhancement in detection sensitivity. Conclusions: Detection of BTx-A antigens by immuno-PCR demonstrated 100% sensitivity and 100% specificity in 100-fold magnitude below the detection limit of ELISA. Significance and Impact of the Study: It is concluded that the immuno-PCR method could be used to detect a very low level of BTx-A for clinical diagnosis.

L8 ANSWER 7 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN
2000:742256 Document No. 133:295361 Clostridium difficile recombinant toxin A repeating units as a carrier protein for **conjugate** vaccines. Wilkins, Tracy D.; Lylerly, David M.; Moncrief, J. Scott; Pavliakova, Danka; Scheerson, Rachel; Robbins, John B. (Techlab, Inc., USA; United States Dept. of Health and Human Services). PCT Int. Appl. WO 2000061761 A2 20001019, 45 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,

LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US9523 20000410. PRIORITY: US 1999-PV128686 19990409; US 2000-PV186201 20000301.

AB The present invention provides for immunogenic compns. and their methods of use as vaccines and their method of preparation These immunogenic compns. comprise a recombinant protein of toxin A of Clostridium difficile conjugated to a polysaccharide of a microbial pathogen. The immunogenic compns. may include only a nontoxic truncated portion of toxin A, particularly the repeating units (rARU), that is conjugated to a microbial pathogen polysaccharide. The yields of these polysaccharide-protein **conjugates** can be significantly increased by prior treatment of rARU with succinic anhydride. Such compns. are effective in eliciting T-cell dependent and antibody responses, and immune responses to pneumococcal type 14, Escherichia coli K1, and Shigella flexneri type 2a polysaccharides in mice are demonstrated. All **conjugates** elicited high levels of serum IgG both to the polysaccharides and to CDTA. These compns. are therefore effective as vaccines for humans, particularly children, and animals in affording protection against one or more microbial pathogens.

L8 ANSWER 8 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN

2000:381824 Document No. 133:22430 **Conjugate** for enrichment in neural cells. Loehr, Achim; Schwab, Manfred (Deutsches Krebsforschungszentrum Stiftung des Oeffentlichen Rechts, Germany). Ger. Offen. DE 19856052 A1 20000608, 12 pp. (German). CODEN: GWXXBX. APPLICATION: DE 1998-19856052 19981204.

AB An active substance is introduced specifically into neurons by conjugating it with the heavy chain of a **Clostridium toxin** in the absence of the toxic light chain. The heavy chain and its fragments, modifications, and fusion proteins mediate neuron-specific binding, uptake, and release of the **conjugate** into the cytoplasm, but are nontoxic. The active agent is coupled to an SH group on the heavy chain through a linker via an SS group; alternatively, if the active agent is a nucleic acid, it is bound via H bonds to an oligonucleotide coupled to the heavy chain, or via ionic bonds to a peptide or protein recombinantly fused to the heavy chain. Use of the heavy chain is facilitated by modifying it to mimic the structure of the native toxin; this is done by substituting other, nontoxic proteins for the light chain and binding them to the heavy chain via SS groups. The **conjugates** can be used for diagnosis and/or therapy of neuronal diseases, e.g. neuroblastoma. Thus, the gene for tetanus toxin heavy chain was cloned, fused with the gene for HMG-1 (high-mobility-group) protein (which nonspecifically binds DNA), and expressed in Escherichia coli.

L8 ANSWER 9 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN

2000:213602 Document No. 133:3463 Clostridium difficile recombinant toxin A repeating units as a carrier protein for **conjugate** vaccines: studies of pneumococcal type 14, Escherichia coli K1, and Shigella flexneri type 2a polysaccharides in mice. Pavliakova, Danka; Moncrief, J. Scott; Lyerly, David M.; Schiffman, Gerald; Bryla, Dolores A.; Robbins, John B.; Schneerson, Rachel (National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, 20892, USA). Infection and Immunity, 68(4), 2161-2166 (English) 2000. CODEN: INFIBR. ISSN: 0019-9567. Publisher: American Society for Microbiology.

AB Unlike the native protein, a nontoxic peptide (repeating unit of the native toxin designated rARU) from Clostridium difficile toxin A (CDTA) afforded an antigen that could be bound covalently to the surface polysaccharides of pneumococcus type 14, Shigella flexneri type 2a, and Escherichia coli K1. The yields of these polysaccharide-protein **conjugates** were significantly increased by prior treatment of rARU with succinic anhydride. **Conjugates**, prepared with rARU or succinylated (rARUsucc), were administered to mice by a clin. relevant

dosage and immunization scheme. All **conjugates** elicited high levels of serum IgG both to the polysaccharides and to CDTA. **Conjugate**-induced anti-CDTA had neutralizing activity in vitro and protected mice challenged with CDTA, similar to the rARU alone. **Conjugates** prepared with succinylated rARU, therefore, have potential for serving both as effective carrier proteins for polysaccharides and for preventing enteric disease caused by C. difficile.

L8 ANSWER 10 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN
1999:763877 Document No. 132:466 Treatment of C. difficile toxin B associated conditions. Armstrong, Glen D.; Heerze, Louis D. (Synsorb Biotech, Inc., Can.). PCT Int. Appl. WO 9961031 A1 19991202, 54 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-CA484 19990527. PRIORITY: US 1998-85032 19980528.

AB This invention relates to prevention and/or treatment of antibiotic associated diarrhea, including Clostridium difficile associated diarrhea (CDAD), pseudomembranous colitis (PMC) and other conditions associated with C. difficile infection, using oligosaccharide compns. which bind C. difficile toxin B. More specifically, the invention concerns neutralization of C. difficile toxin B associated with such conditions.

L8 ANSWER 11 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN
1999:360160 Document No. 131:180860 Development and evaluation of various enzyme-linked immunosorbent assays for the detection of Clostridium perfringens β anti-toxins. Krt, Brane (Gerbiceva 60, Veterinary faculty, Institute for Microbiology and Parasitology, University of Ljubljana, Ljubljana, 1115, Slovenia). FEMS Immunology and Medical Microbiology, 24(3), 293-297 (English) 1999. CODEN: FIMIEV. ISSN: 0928-8244. Publisher: Elsevier Science B.V..

AB The aim of our work was to develop an ELISA for the detection of antibodies against the Clostridium perfringens β toxin. For this purpose, five different ways of performing an ELISA were investigated. Pos. and neg. sera of different animals and partially purified β toxin were used. In all ELISA tests, microplates were first coated with monoclonal antibodies against the C. perfringens β toxin. Actually, the first three ways of performing ELISA proved to be an inhibition or a blocking ELISA. In the first of these modifications, the examined serum was added on a microplate after the toxin. In the second two tests, they were added simultaneously after they were incubated together (60 min at room temperature or overnight at 4°, resp.). An anti-toxin **conjugate** was used for the detection. It was also used in a competitive ELISA, where it was added together with the examined serum on the microplate, to which the toxin was already bound. The fifth way of performing an ELISA differed from others by the use of conjugated anti-species Ig for the detection. The biggest differences in absorbances between pos. and neg. sera were found in the blocking ELISA, where the mixture of the toxin and the examined serum were previously incubated overnight at 4°. The smallest differences in absorbance were found when anti-species **conjugates** were used.

L8 ANSWER 12 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN
1998:682608 Document No. 129:313112 Methods for concentrating and detecting ligands using magnetic particles. Valkirs, Gunars E. (Biosite Diagnostics, Inc., USA). PCT Int. Appl. WO 9845684 A1 19981015, 49 pp. DESIGNATED STATES: W: CA, JP; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US6605 19980403. PRIORITY: US 1997-44292 19970404.

AB This invention provides methods, compns. and kits for concentrating target ligands, including microorganisms, from samples, including biol. samples. The methods involve the use of magnetic particles to concentrate the target analytes. Also provided are methods, compns. and kits for detecting the presence of target ligands in samples. A high-sensitivity assay for Clostridium difficile toxin A used magnetic beads to concentrate the toxin before detecting the toxin by sandwich ELISA. Preparation of the monoclonal antibodies and reagents for the separation and assay are described.

L8 ANSWER 13 OF 25 EMBASE
on STN
95099755 EMBASE Document No.: 1995099755. Immunological detection of Clostridium botulinum toxin type A in therapeutic preparations. Ekong T.A.N.; McLellan K.; Sesardic D.. Division of Bacteriology, Nat. Inst. Biol. Standards/Control, South Mimms,Potters Bar, Hertfordshire EN6 3QG, United Kingdom. Journal of Immunological Methods 180/2 (181-191) 1995. ISSN: 0022-1759. CODEN: JIMMBG. Pub. Country: Netherlands. Language: English. Summary Language: English.
AB The potent neurotoxins produced by strains of Clostridium botulinum act by blocking the release of acetylcholine from peripheral nerve junctions. This specific action of the botulinum neurotoxins is now being exploited therapeutically to treat a variety of conditions involving involuntary muscle spasms. We aimed to develop a sensitive and specific enzyme-linked immunosorbent assay (ELISA) which may be used in parallel with the currently accepted mouse bioassay test for the determination of botulinum neurotoxin type A in therapeutic preparations. High titre polyclonal antitoxins and their biotin derivatives, highly labelled horseradish peroxidase (HRP)-antibody **conjugates**, and streptavidin-biotin-HRP complexes were prepared and used in a sandwich ELISA for the detection of pure neurotoxin and neurotoxin in therapeutic material. The ELISA utilized either a monoclonal or polyclonal antibody as capture agent and HRP-labelled IgG or F(ab'), fragment as second antibody. The limit of detection was 4-8 pg of purified toxin/ml (gcv < 13%), equivalent to 1-2 mouse bioassay units/ml. The assay was used to evaluate therapeutic preparations and the results compared with the mouse bioassay. The lower limit of detection for a therapeutic preparation of BoTxA was 2-5 mouse bioassay units/ml. Although across different manufacturers and bulk products there was no correlation between immunologically detected neurotoxin and its biological activity in different therapeutic preparations ($r = -0.44$, $p = 0.34$, $n = 8$), the assay could be used to quantify neurotoxin in therapeutic preparations derived from the same bulk concentrate and manufacturer. The assay is relatively simple, and may be readily adapted to routine monitoring of BoTxA. content in therapeutic preparations.

L8 ANSWER 14 OF 25 CAPLUS
1997:290501 Document No. 126:325016 Synthesis and cytotoxic activity of immuno-toxins on the basis of bacterial toxin of phospholipase c. Limareva, T. D.; Plotnikov, V. M.; Kusmartsev, S. A.; Ermakov, G. F.; Zemlyanskaya, N. V. (NIL Eksp. Bio-Med. Modelirovaniya TNTs, Tomsk NII Vaktsin Syvorotok, Tomsk, Russia). Immunologiya (Moscow) (5), 60-62 (Russian) 1995. CODEN: IMUNDA. ISSN: 0206-4952. Publisher: Meditsina.
AB Immunotoxins are compds. composed of specific antibodies which perform vector function and toxin having killer function. Using soluble carbodiimide, we obtained immunotoxins on the basis of polyclonal antibodies to K-562 human erythroleukemia cells, monoclonal antibodies to mouse antigen of erythroblasts and phospholipase C from Cl. Perfringens t.A. The **conjugates** were purified by means of tel-chromatog. and ion-exchange chromatog. The antigenic, enzymic, and cytotoxic characteristics of the above immunotoxins demonstrated that the prepns. have high cytotoxic and specific activity.

L8 ANSWER 15 OF 25 CAPLUS
1995:277178 Document No. 122:46474 Therapeutic use of Clostridium difficile toxin A. Carroll, Sean B.; Firca, Joseph R.; Kushnaryov, Vladimir M.;

Redlich, Philip N.; Grossberg, Sidney E.; Sedmak, J. James (Ophidian Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 9424155 A1 19941027, 27 pp. DESIGNATED STATES: W: AU, CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US3765 19940406. PRIORITY: US 1993-44631 19930408.

AB Methods for treating cancer are described, including methods for treating colon and pancreatic cancer. Bacterial toxins and portions of bacterial toxins are employed as both diagnostic and therapeutic agents. A suitable peptide may contain the sequence CQTIDGKKYYFN-NH2, the cysteine residue being useful for conjugating the peptide to proteins such as ricin. Methods for purifying the toxin are presented, along with procedures for determining sensitivity of various human tumor cell lines to bacterial toxins.

L8 ANSWER 16 OF 25 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

94327878 EMBASE Document No.: 1994327878. Preparation, characterization, and immunological properties in mice of Escherichia coli O157 O-specific polysaccharide-protein **conjugate** vaccines. Konadu E.; Robbins J.B.; Shiloach J.; Bryla D.A.; Szu S.C.. Developmental/Molec. Immunity Lab., NICHHD,Bethesda, MD 20892, United States. Infection and Immunity 62/11 (5048-5054) 1994.
ISSN: 0019-9567. CODEN: INFIBR. Pub. Country: United States. Language: English. Summary Language: English.

AB Escherichia coli O157 causes severe enteritis and the extraintestinal complication of hemolytic-uremic syndrome, with their highest incidence occurring in children. We postulated that serum immunoglobulin G (IgG) antibodies to the O-specific polysaccharide of lipopolysaccharide (LPS) may confer protective immunity to enteric pathogens by inducing bactericidal reactions against the ingested organisms in the jejunum (J. B. Robbins, C. Chu, and R. Schneerson, Clin. Infect. Dis. 15:346-361, 1992; S. C. Szu, R. Gupta, and J. B. Robbins, p. 381-394, in I. K. Wachsmuth, P. A. Blake, and O. Olsvik, ed., Vibrio cholerae, 1994). Because polysaccharide-protein **conjugates** induce serum IgG antibodies in infants, we bound the O-specific polysaccharide of E. coli O157 to proteins. E. coli O157 LPS, treated with acetic acid or hydrazine, was derivatized with adipic acid dihydrazide and bound to proteins by carbodiimide-mediated condensation. **Conjugates** of these adipic hydrazide derivative were prepared with bovine serum albumin, formalin-treated exotoxin C of Clostridium welchii (Pig Bel toxoid), or Pseudomonas aeruginosa recombinant exoprotein A. The **conjugates** had low levels of endotoxin and elicited serum antibodies with bactericidal activity to the O157 LPS. The largest increase in LPS antibodies was of the IgG class. Clinical evaluation of E. coli O157-toxoid **conjugates** is planned.

L8 ANSWER 17 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN

1994:242760 Document No. 120:242760 An ELISA for detection of botulinal toxin types A, B, and E in inoculated food samples. Potter, Marianne D.; Meng, Jianghong; Kimsey, Paul (Westreco/Nestle, New Milford, CT, 06776, USA). Journal of Food Protection, 56(10), 856-61 (English) 1993. CODEN: JFPRDR. ISSN: 0362-028X.

AB An ELISA was developed to screen for the presence of botulinal toxin types A, B, and E in inoculated food studies. A com. available trivalent antitoxin (Connaught Labs., Ontario) was used as a capture antibody and biotinylated for use as a secondary antibody. An avidin-alkaline phosphatase **conjugate** coupled with an enzyme-based amplification system resulted in a high degree of sensitivity. Detection levels of purified neurotoxins in gelatin phosphate buffer were 9 LD50 for type A and <1 i.p. mouse LD50 for types B and E, resp. Toxin produced by two-type F strains (proteolytic and nonproteolytic) was detected in a liquid laboratory medium. In a comparative study of over 490 samples of ground turkey meat inoculated with C. botulinum types E and nonproteolytic B, the ELISA gave no false negatives and 91 false positives. False positives were thought to be due to the presence of inactivated toxin or toxin levels insufficient to cause

mouse death. Statistical anal. of these data showed an ELISA sensitivity of 100%, specificity of 70.6%, and an efficiency of 81.4% when compared to the mouse bioassay for detection of botulinal toxins types B and E. Coffee intermediates inoculated with proteolytic Clostridium botulinum types A and B caused nonspecific death in mice but were neg. for presence of toxin by ELISA.

L8 ANSWER 18 OF 25 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 1

93127021 EMBASE Document No.: 1993127021. ADP-ribosylation of Drosophila indirect-flight-muscle actin and arthrin by Clostridium botulinum C2 toxin and Clostridium perfringens iota toxin. Just I.; Hennessey E.S.; Drummond D.R.; Aktories K.; Sparrow J.C.. Inst Pharmakologie und Toxikologie, Universitat des Saarlandes,D-Homburg, Germany. Biochemical Journal 291/2 (409-412) 1993.
ISSN: 0264-6021. CODEN: BIJOAK. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Purified Drosophila indirect-flight-muscle actin and arthrin, an actin-ubiquitin **conjugate**, were ADP-ribosylated by Clostridium botulinum C2 toxin and Clostridium perfringens iota toxin. Phalloidin treatment inhibited the ADP-riboyslation of Drosophila actin and arthrin. Like actin, the ADP-ribosearthrin linkage was sensitive towards hydroxylamine treatment, indicating arginine as the amino acid acceptor. Actin translated in vitro from the indirect-flight-muscle-specific gene Act88F was ADP-ribosylated by C. botulinum C2 toxin and C. perfringens iota toxin. Actin from the R177Q mutant of Act88F translated in vivo was not ADP-ribosylated confirming Arg-177 as the ADP-ribose acceptor. Mutant L176M actin was modified by both toxins, indicating that amino acid 176 of actin does not define the substrate specificity of C. botulinum C2 toxin. Whereas the gene products of various C-terminal mutants of Act88F translated in vitro (E334K, V339I, E364K, G368E, R372H) were substrates for ADP-ribosylation by C. botulinum C2 toxin and by C. perfringens iota toxin, neither toxin modified the N-terminal O-12 deletion mutant.

L8 ANSWER 19 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN

1992:250274 Document No. 116:250274 Detection, isolation, and purification of Clostridium difficile toxin A with toxin receptors. Wilkins, Tracy D.; Tucker, Kenneth D. (Virginia Tech Intellectual Properties, Inc., USA). U.S. US 5098826 A 19920324, 15 pp. (English). CODEN: USXXAM. APPLICATION: US 1990-491396 19900309.

AB C. difficile toxin A is detected by contacting a specimen with a reagent containing the human X (Lex), Y (Ley), or I antigens, each of which is a specific receptor for toxin A. Binding of toxin A is determined by ELISA, latex agglutination, or crossed immunoelectrophoresis. The method may also be used to isolate and purify toxin A. Conversely, immobilized toxin A may be used to detect, isolate, or purify biol. materials expressing the X, Y, or I antigens.

L8 ANSWER 20 OF 25 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

92270676 EMBASE Document No.: 1992270676. A conjugated synthetic peptide corresponding to the C-terminal region of Clostridium perfringens type A enterotoxin elicits an enterotoxin- neutralizing antibody response in mice. Mietzner T.A.; Kokai-Kun J.F.; Hanna P.C.; McClane B.A.. Molec. Genetics/Biochemistry Dept., Pittsburgh Univ. School of Medicine,Pittsburgh, PA 15261-2072, United States. Infection and Immunity 60/9 (3947-3951) 1992.
ISSN: 0019-9567. CODEN: INFIBR. Pub. Country: United States. Language: English. Summary Language: English.

AB A synthetic peptide homolog corresponding to the C-terminal 30 amino acids of Clostridium perfringens type A enterotoxin (CPE) was conjugated to a thyroglobulin carrier and used to immunize mice. **Conjugate** -immunized mice produced antibodies which neutralized native CPE cytotoxicity, at least in part, by blocking enterotoxin binding. This peptide may be useful for the development of a vaccine to protect against

CPE-mediated disease.

L8 ANSWER 21 OF 25 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
92361774 EMBASE Document No.: 1992361774. A novel membrane-acting immunotoxin, the immunolysin, with therapeutic potential. Drobniewski F.A.; Watson G.J.; Wawrzynczak E.J.; Alouf J.E.; Thorpe P.E.. Drug Targeting Laboratory, ICRF Laboratories,Lincolns Inn Fields, London WC2A 3PX, United Kingdom. Biochemical Society Transactions 20/4 (318S) 1992. ISSN: 0300-5127. CODEN: BCSTB5. Pub. Country: United Kingdom. Language: English.

L8 ANSWER 22 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN
1988:185113 Document No. 108:185113 Vaccines based on antigen coupled to insoluble supports. Wilkins, Tracy D.; Lyerly, David M. (Research Corp., USA). U.S. US 4713240 A 19871215, 6 pp. (English). CODEN: USXXAM. APPLICATION: US 1985-719775 19850404.
AB A vaccine composition useful in initiating an immune response against e.g. Clostridium comprises an immunogenically effective amount of ≥1 nonenzymic toxin of the organism covalently bonded or crosslinked to a water-insol. support, in combination with a pharmaceutically acceptable carrier. Toxin A of C. difficile was covalently bound to Affi-Gel 10 beads, remaining active sites on the beads were blocked by addition of ethanolamine, and unbound antigen was washed off with phosphate-buffered saline. BALB/C mice were injected i.p. with 15 μg of the toxin A-Affi-Gel 10 vaccine or 100 μg toxoid A once every week for 2 mo. Both groups showed similar levels of toxin A antibodies. None of the mice treated with the toxin died.

L8 ANSWER 23 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN
1984:524544 Document No. 101:124544 Interaction of Clostridium difficile toxin A and L cells in culture. Shahrabadi, M. S.; Bryan, L. E.; Lee, P. W. K. (Health Sci. Cent., Univ. Calgary, Calgary, AB, T2N 4N1, Can.). Canadian Journal of Microbiology, 30(7), 874-83 (English) 1984. CODEN: CJMIAZ. ISSN: 0008-4166.
AB Toxin A of C. difficile was conjugated to ferritin and applied to L cells to localize binding sites of this toxin to the cell surface. The toxin A **conjugate** attached to the cell membrane in aggregated form. Toxin A was found inside the cytoplasm 6 h after cell treatment, mainly in the form of aggregates inside the cytoplasmic vacuoles. At 24 h after exposure, toxin A could be detected within the cytoplasm. Tunicamycin treatment of cells reduced the cell-binding efficiency of toxin A to 50%, but neuraminidase did not effect toxin binding significantly.

L8 ANSWER 24 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN
1984:587867 Document No. 101:187867 Quantitative determination of Clostridium botulinum toxin by reaction with enzyme antibodies. Nikiforov, V. N.; Kremlev, G. I.; Etkin, A. F.; Ermolin, G. A.; Nikiforov, V. V. (Tsentr. Inst. Usoversh. Vrachei, Moscow, USSR). Eksperimental'naya Meditsina (Riga), 18, 150-6 (Russian) 1984. CODEN: EKMEDL.
AB Enzyme-linked immunosorbent assay (ELISA) was used for rapid detection and identification of botulin toxin types A, B, and E. Horseradish peroxidase were used for preparing enzyme **conjugates.** Specific antiserums were raised in rabbits. The potency of the toxins was determined in mice. Factor affecting antigen-antibody reactions are detailed.

L8 ANSWER 25 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN
1978:437455 Document No. 89:37455 Enzyme-linked immunosorbent assay for detection of Clostridium botulinum toxin type A. Notermans, Serve; Dufrenne, John; Van Schothorst, Mike (Lab. Zoonoses Food Microbiol., Natl. Inst. Public Health, Bilthoven, Neth.). Japanese Journal of Medical Science & Biology, 31(1), 81-5 (English) 1978. CODEN: JJMCAQ. ISSN: 0021-5112.
AB Botulinum toxin type A was determined using enzyme-linked immunosorbent assay (ELISA). Polystyrene tubes were coated with horse serum against botulinum

toxin type A and incubated; the tubes were washed and toxin samples were added and incubated again. The adsorbed toxin was labeled with rabbit serum against botulinum toxin A and the amount of rabbit serum adsorbed was measured with sheep antirabbit serum **conjugate** with horse radish peroxidase following incubation and addition of the substrate 5-aminosalicylic acid; the reaction product (brown color) was measured spectrophotometrically at 449 nm. The method allowed detection of 50-100 mouse i.p. LD50 of the toxin type A; no cross reaction occurred with other types of botulinum toxins tested.

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of L-numbers, enter DISPLAY HISTORY at an arrow prompt (=>).

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L9           58 L3 AND FUSION

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L10          53 DUP REMOVE L9 (5 DUPLICATES REMOVED)

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L10  ANSWER 1 OF 53  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
2005002704  EMBASE  Bacteria and bacterial toxins as therapeutic agents for
     solid tumors. Michl P.; Gress T.M.. T.M. Gress, Abt. Innere Medizin I,
     Universitatsklinikum Ulm, Robert-Koch-Str. 8, 89081 Ulm, Germany.
     thomas.gress@medizin.uni-ulm.de. Current Cancer Drug Targets  4/8
     (689-702)  2004.
     Refs: 116.
     ISSN: 1568-0096. CODEN: CCDTB. Pub. Country: Netherlands. Language:
     English. Summary Language: English.
AB   Patients with advanced solid tumors frequently relapse and succumb to
     their metastatic disease after developing resistance to conventional
     treatment modalities such as chemotherapy and radiotherapy. In these
     patients, novel strategies of targeting widespread tumors are urgently
     needed. The increasing knowledge of the underlying pathogenetic mechanisms
     has led to the identification of numerous molecules that are overexpressed
     in various tumors and accumulate at the cell surface. The use of
     genetically modified bacteria and their toxins targeting these surface
     molecules has emerged as a promising new treatment strategy in refractory
     cancers. This review focuses on bacterial toxins such as Diphtheria toxin
     (DT), Pseudomonas exotoxin A (PE) and Clostridium perfringens enterotoxin
     (CPE). In addition, the use of anaerobic bacteria such as Clostridium,
     Salmonella and Bifidobacterium spp. as drug-delivery systems targeting
     hypoxic tumor areas will be discussed as a new therapeutic modality of
     advanced solid tumors.

L10  ANSWER 2 OF 53  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
2004257528  EMBASE  Requirement for the Rac GTPase in Chlamydia trachomatis
     invasion of non-phagocytic cells. Carebeo R.A.; Grieshaber S.S.; Hasenkrug
     A.; Dooley C.; Hackstadt T.. T. Hackstadt, Host-Parasite Interfections
     Section, Lab. of Intracellular Parasites, NIAID, Hamilton, MT 59840,
     United States. THACKSTADT@niaid.nih.gov. Traffic  5/6 (418-425)  2004.
     Refs: 47.
     ISSN: 1398-9219. CODEN: TRAFFA. Pub. Country: Denmark. Language: English.
     Summary Language: English.
AB   Chlamydiae are gram-negative obligate intracellular pathogens to which
     access to an intracellular environment is paramount to their survival and
     replication. To this end, chlamydiae have evolved extremely efficient
     means of invading nonphagocytic cells. To elucidate the host cell
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machinery utilized by Chlamydia trachomatis in invasion, we examined the roles of the Rho GTPase family members in the internalization of chlamydial elementary bodies. Upon binding of elementary bodies on the cell surface, actin is rapidly recruited to the sites of internalization. Members of the Rho GTPase family are frequently involved in localized recruitment of actin. Clostridial Toxin B, which is a known enzymatic inhibitor of Rac, Cdc42 and Rho GTPases, significantly reduced chlamydial invasion of HeLa cells. Expression of dominant negative constructs in HeLa cells revealed that chlamydial uptake was dependent on Rac, but not on Cdc42 or RhoA. Rac but not Cdc42 was found to be activated by chlamyclial attachment. The effect of dominant negative Rac expression on chlamydial uptake is manifested through the inhibition of actin recruitment to the sites of chlamydial entry. Studies utilizing Green Fluorescent Protein **fusion** constructs of Rac, Cdc42 and RhoA, showed Rac to be the sole member of the Rho GTPase family recruited to the site of chlamydial entry. Copyright .COPYRGT. Blackwell Munksgaard 2004.

L10 ANSWER 3 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN

2003:696462 Document No. 139:207733 Construction of recombinant single-chain toxins for use in vaccines and toxin assays. Shone, Clifford Charles; Quinn, Conrad Padraig; Foster, Keith Alan; Chaddock, John; Marks, Philip; Sutton, J. Mark; Stancombe, Patrick; Wayne, Jonathan (Microbiological Research Authority, UK; Speywood Laboratory Limited). U.S. Pat. Appl. Publ. US 2003166238 A1 20030904, 37 pp., Cont.-in-part of U.S. Ser. No. 255,829. (English). CODEN: USXXCO. APPLICATION: US 2002-241596 20020912. PRIORITY: GB 1996-17671 19960823; GB 1996-25996 19961213; US 1996-782893 19961227; WO 1997-GB2273 19970822; US 1999-255829 19990223; US 1999-242689 19990223.

AB A single-chain polypeptide is provided which comprises first and second domains. The first domain enables the polypeptide to cleave one or more vesicle or plasma-membrane associated proteins essential to exocytosis, and the second domain enables the polypeptide to be translocated into a target cell or increases the solubility of the polypeptide, or both. The polypeptide thus combines useful properties of a clostridial toxin, such as a botulinum or tetanus toxin, without the toxicity associated with the natural mol. The polypeptide can also contain a third domain that targets it to a specific cell, rendering the polypeptide useful in inhibition of exocytosis in target cells. **Fusion** proteins comprising the polypeptide, nucleic acids encoding the polypeptide and methods of making the polypeptide are also provided. Controlled activation of the polypeptide is possible and the polypeptide can be incorporated into vaccines and toxin assays.

L10 ANSWER 4 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2003219696 EMBASE Mutational analysis of the enzymatic domain of Clostridium difficile toxin B reveals novel inhibitors of the wild-type toxin. Spyres L.M.; Daniel J.; Hensley A.; Qa'Dan M.; Ortiz-Leduc W.; Ballard J.D.. J.D. Ballard, Department of Botany, University of Oklahoma, 770 Van Vleet Oval, Norman, OK 73019, United States. jballard@ou.edu. Infection and Immunity 71/6 (3294-3301) 1 Jun 2003.
Refs: 31.
ISSN: 0019-9567. CODEN: INFIBR. Pub. Country: United States. Language: English. Summary Language: English.

AB Toxin B (TcdB), a major Clostridium difficile virulence factor, glucosylates and inactivates the small GTP-binding proteins Rho, Rac, and Cdc42. In the present study we provide evidence that enzymatically inactive fragments of the TcdB enzymatic domain are effective intracellular inhibitors of native TcdB. Site-directed and deletion mutants of the TcdB enzymatic region (residues 1 to 556), lacking receptor binding and cell entry domains, were analyzed for attenuation of glucosyltransferase and glucosylhydrolase activity. Five of six derivatives from TcdB(1-556) were found to be devoid of enzymatic activity. In order to facilitate cell entry, mutants were genetically fused to lfn, which encodes the protective antigen binding region of

anthrax toxin lethal factor and mediates the cell entry of heterologous proteins. In line with reduced enzymatic activity, the mutants also lacked cytotoxicity. Remarkably, pretreatment or cotreatment of cells with four of the mutants provided protection against the cytotoxic effects of native TcdB. Furthermore, a CHO cell line expressing enzymatically active TcdB(1-556) was also protected by the mutant-derived inhibitors, suggesting that inhibition occurred at an intracellular location. Protection also was afforded by the inhibitor to cells treated with Clostridium sordellii lethal toxin (TcsL), which uses the same cosubstrate as TcdB but shares Rac only as a common substrate target. Finally, the inhibitor did not provide protection against Clostridium novyi alpha-toxin (Tcnα), which shares similar substrates with TcdB yet uses a different cosubstrate. This is the first report to demonstrate that the potential exists to inhibit toxins at their intracellular site of action by using inactive mutants.

L10 ANSWER 5 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2003219777 EMBASE SNARE function revisited. Rizo J.. J. Rizo, Department of Biochemistry, Univ. of TX Southwestern Med. Ctr., Dallas, TX 75390, United States. jose@arnie.swmed.edu. Nature Structural Biology 10/6 (417-419) 1 Jun 2003.
Refs: 30.
ISSN: 1072-8368. CODEN: NSBIEW. Pub. Country: United States. Language: English. Summary Language: English.

AB The notion that SNARE proteins constitute the minimal machinery for intracellular membrane **fusion** has been accepted by many scientists. An EPR study now brings new structural data on the neuronal SNARE VAMP-2 and emphasizes the importance of rigorous evidence before a hypothesis becomes a dogma.

L10 ANSWER 6 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2004132767 EMBASE Construction of a **fusion** protein carrying antigenic determinants of enteric clostridial toxins. Belyi I.F.; Varfolomeeva N.A.. I.F. Belyi, Laboratory of Molecular Pathogenesis, Gamaleya Res. Inst. Epidemiol. M., ulitsa Gamalei 18, Moscow 123098, Russian Federation. belyi@riem.ru. FEMS Microbiology Letters 225/2 (325-329) 29 Sep 2003.
Refs: 24.
ISSN: 0378-1097. CODEN: FMLED7.
Publisher Ident.: S 0378-1097(03)00560-3. Pub. Country: Netherlands. Language: English. Summary Language: English.

AB Clostridium difficile and Clostridium perfringens type A are infectious agents of enteric diseases. The main virulence factors of these microorganisms include toxins A and B of C. difficile (ToxA and ToxB) and enterotoxin of C. perfringens (Cpe). In this study genetic constructions have been created for the expression of ToxA, ToxB and Cpe fragments either as individual components or as a hybrid multidomain (ToxA-ToxB-Cpe) protein. Rabbit monospecific sera raised against individual peptides reacted with the chimeric product indicating that the corresponding antigenic determinants were correctly expressed on the hybrid molecule. Furthermore, mice immunized with the **fusion** protein produced antibodies specific to each of the three separate components. These data suggest that the constructed three-domain molecule could be used in future studies for development of a vaccine against enteric clostridial diseases.

L10 ANSWER 7 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN

2003:790100 Document No. 140:176405 Molecular cloning, overexpression in Escherichia coli, and purification of 6+ his-tagged C-terminal domain of Clostridium difficile toxins A and B. Letourneur, Odile; Ottone, Sophie; Delauzun, Vincent; Bastide, Marie-Claire; Foussadier, Agnes (BioMerieux, Chemin de l'Orme, Marcy l'Etoile, 69280, Fr.). Protein

Expression and Purification, 31(2), 276-285 (English) 2003. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Elsevier Science.

AB Genomic DNA from ribotype-01 and -17 Clostridium difficile strains was used for amplification of the sequences encoding the carboxy-terminal domain of toxins A (TcdA) and B (TcdB). The deduced C-terminal TcdB ribotype-01 and -17 domains share 99.5% amino acid sequence identity while TcdA ribotype-17 comprises a 607 amino acid deletion compared to TcdA-01. When compared to previously sequenced C. difficile toxins, 99.3% amino acid identity was found between TcdA-01 and TcdA from strain VPI10643 and 98.8% identity between TcdA-17 and TcdA from strain F-1470. The obtained sequences were fused in 3' to a sequence encoding a hexahistidine tag and cloned into an Escherichia coli expression vector. The recombinant proteins were expressed in E. coli and purified using single-step metal-chelate chromatog. The recombinant carboxy-terminal domain of TcdA-01 was purified from the soluble E. coli lysate fraction whereas TcdA-17 and TcdB-17 carboxy-terminal domains were purified from inclusion bodies. At least 40 mg of each protein was purified per L of bacterial culture. The recombinant toxin domains were detected specifically by Western blot and ELISA with antibodies against native C. difficile toxins. This study demonstrated that the carboxy-terminal domains of TcdA and TcdB can be produced using an E. coli expression system and easily purified. These recombinant, stable, and non-toxic proteins provide a convenient source for use in the diagnosis of C. difficile infections, instead of native toxins, as controls and calibrators in immunoassay kits and to obtain specific monoclonal antibodies.

L10 ANSWER 8 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2003320971 EMBASE Revisiting the role of SNAREs in exocytosis and membrane **fusion.** Szule J.A.; Coorssen J.R.. J.R. Coorssen, Dept. of Physiology and Biophysics, Faculty of Medicine, University of Calgary, Calgary, Alta. T2N 4N1, Canada. jcoorsse@ucalgary.ca. Biochimica et Biophysica Acta - Molecular Cell Research 1641/2-3 (121-135) 18 Aug 2003.
Refs: 209.
ISSN: 0167-4889. CODEN: BAMRDP. Pub. Country: Netherlands. Language: English. Summary Language: English.

AB For over a decade SNARE hypotheses have been proposed to explain the mechanism of membrane **fusion**, yet the field still lacks sufficient evidence to conclusively identify the minimal components of native **fusion.** Consequently, debate concerning the postulated role(s) of SNAREs in membrane **fusion** continues. The focus of this review is to revisit original literature with a current perspective. Our analysis begins with the earliest studies of clostridial toxins, leading to various cellular and molecular approaches that have been used to test for the roles of SNAREs in exocytosis. We place much emphasis on distinguishing between specific effects on membrane **fusion** and effects on other critical steps in exocytosis. Although many systems can be used to study exocytosis, few permit selective access to specific steps in the pathway, such as membrane **fusion.** Thus, while SNARE proteins are essential to the physiology of exocytosis, assay limitations often prevent definitive conclusions concerning the molecular mechanism of membrane **fusion.** In all, the SNAREs are more likely to function upstream as modulators or priming factors of **fusion.** .COPYRGT. 2003 Elsevier B.V. All rights reserved.

L10 ANSWER 9 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN

2002:927279 Document No. 138:29103 Pharmaceutical use for secreted bacterial effector proteins. Sutton, John Mark; Shone, Clifford Charles (Microbiological Research Authority, UK). PCT Int. Appl. WO 2002096467 A2 20021205, 130 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,

UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-GB2384 20020521. PRIORITY: GB 2001-12687 20010524.

AB A polypeptide conjugate contains a bacterial injectable effector protein, secreted by a modified pilus or "needle-like" structure comprising a type III or type IV secretion apparatus, and a carrier that targets the conjugate to a target cell. The effector protein is used for a variety of purposes including treatment of neurodegenerative disease, intracellular infection and diseases associated with defects of secretion.

L10 ANSWER 10 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN

2002:504638 Document No. 137:57595 Use of a composition containing a transporter and a **fusion** protein for the stimulation of nerve growth, the inhibition of scar tissue formation, the reduction of secondary damage and/or the accumulation of macrophages. Monnier, Philippe P.; Mueller, Bernhard K.; Schwab, Jan (Migragen A.-G., Germany). PCT Int. Appl. WO 2002051429 A2 20020704, 40 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (German). CODEN: PIXXD2. APPLICATION: WO 2001-EP15147 20011220. PRIORITY: DE 2000-10064195 20001222.

AB The invention relates to the use of a composition, comprising a **fusion** protein and at least one transporter for the in-vivo inhibition of scar tissue formation, the in-vivo reduction of secondary damage and/or the in-vivo accumulation of macrophages. The **fusion** protein contains at least one binding domain for the transporter and at least one modulation domain for the covalent modification of small GTP-binding proteins. The transporter permits the uptake of the **fusion** protein in a target cell.

L10 ANSWER 11 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN

2002:256064 Document No. 136:293508 New immunogenic complex comprising glycoside, lipid, iscom matrix, antigen and enzyme. Lycke, Nils; Dalsgaard, Kristian; McMowat, Allan; Loewenadler, Bjoern; Kaastrup, Peter (Isconova Ab, Swed.). PCT Int. Appl. WO 2002026255 A1 20020404, 64 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-SE2117 20011001. PRIORITY: SE 2000-3538 20000929.

AB The invention relates to an immunogenic complex comprising at least one glycoside and at least one lipid, integrated into an iscom complex or matrix, and at least one antigen which antigen is integrated into the iscom complex or coupled on to or mixed with the iscom complex or iscom matrix complex, characterized in that it also comprises at least one enzyme. The enzyme confers ADP-ribosylating activity and is derived from cholera toxin, Escherichia coli heat labile enterotoxin, pertussis toxin, **Clostridia toxin**, Shigella toxin or pseudomonas toxin.
It also relates to such a complex comprising at least one peptide which specifically binds to a receptor expressed on a cell capable of antigen presentation, which cell expresses MHC Class I or Class II and to compns. comprising the complexes. The antigen presenting cell is selected from lymphocytes, macrophages, dendritic cells, Langerhans cells and epithelial cells. The immunogenic complex further comprises other immunomodulatory compds or targeting agents for pharmaceutical and veterinary use.

L10 ANSWER 12 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2003059235 EMBASE Clostridium perfringens iota toxin: Mapping of the Ia domain involved in docking with Ib and cellular internalization. Marvaud J.-C.; Stiles B.G.; Chenal A.; Gillet D.; Gibert M.; Smith L.A.; Popoff M.R.. M.R. Popoff, CNR Anaerobies, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France. mpopoff@pasteur.fr. Journal of Biological Chemistry 277/46 (43659-43666) 15 Nov 2002.
Refs: 52.
ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.

AB Clostridium perfringens iota toxin consists of two unlinked proteins. The binding component (Ib) is required to internalize into cells an enzymatic component (Ia) that ADP-ribosylates G-actin. To characterize the Ia domain that interacts with Ib, **fusion** proteins were constructed between the C. botulinum C3 enzyme, which ADP-ribosylates Rho, and various truncated versions of Ia. These chimeric molecules retained the wild type ADP-ribosyltransferase activity specific for Rho and were recognized by antibodies against C3 enzyme and Ia. Internalization of each chimera into Vero cells was assessed by measuring the disorganization of the actin cytoskeleton and intracellular ADP-ribosylation of Rho. **Fusion** proteins containing C3 linked to the C terminus of Ia were transported most efficiently into cells like wild type Ia in an Ib-dependent manner that was blocked by bafilomycin A1. The minimal Ia fragment that promoted translocation of Ia-C3 chimeras into cells consisted of 128 central residues (129-257). These findings revealed that iota toxin is a suitable system for mediating the entry of heterologous proteins such as C3 into cells.

L10 ANSWER 13 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2002428878 EMBASE Rac GTPase plays an essential role in exocytosis by controlling the **fusion** competence of release sites. Humeau Y.; Popoff M.R.; Kojima H.; Doussau F.; Poulain B.. B. Poulain, Neurotransmiss./Secr. Neuroendocr., Unite Propre de Rech 2356 du Centre, National de Recherche Scientifique, 5, rue Blaise Pascal, F-67084 Strasbourg Cedex, France. poulain@neurochem.u-strasbg.fr. Journal of Neuroscience 22/18 (7968-7981) 15 Sep 2002.
Refs: 84.
ISSN: 0270-6474. CODEN: JNRSDS. Pub. Country: United States. Language: English. Summary Language: English.

AB The role of small GTPases of the Rho family in synaptic functions has been addressed by analyzing the effects of lethal toxin (LT) from Clostridium sordellii strain IP82 (LT82) on neurotransmitter release at evoked identified synapses in the buccal ganglion of Aplysia. LT82 is a large monoglucosyltranferase that uses UDP-glucose as cofactor and glucosylates Rac (a small GTPase related to Rho), and Ras, Ral, and Rap (three GTPases of the Ras family). Intraneuronal application of LT (50 nM) rapidly inhibits evoked acetylcholine (ACh) release as monitored electrophysiologically. Injection of the catalytic domain of the toxin similarly blocked ACh release, but not when key amino acids needed for glucosylation were mutated. Intraneuronal application of competitive nucleotide sugars that differentially prevent glucosylation of Rac- and Ras-related GTPases, and the use of a toxin variant that affects a different spectrum of small GTPases, established that glucosylation of Rac is responsible for the reduction in ACh release. To determine the quantal release parameters affected by Rac glucosylation, we developed a nonstationary analysis of the fluctuations in postsynaptic response amplitudes that was performed before and after the toxin had acted or during toxin action. The results indicate that neither the quantal size nor the average probability for release were affected by lethal toxin action. ACh release blockage by LT82 was only caused by a reduction in the number of functional release sites. This reveals that after docking of synaptic vesicles, vesicular Rac stimulates a membrane effector (or

effectors) essential for the **fusion** competence of the exocytotic sites.

L10 ANSWER 14 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN
2002:881218 Document No. 138:249956 The uptake machinery of clostridial actin ADP-ribosylating toxins - a cell delivery system for **fusion** proteins and polypeptide drugs. Barth, Holger; Bloecker, Dagmar; Aktories, Klaus (Institut fuer Experimentelle und Klinische Pharmakologie und Toxikologie der Albert-Ludwigs-Universitaet Freiburg, Otto-Krayer-Haus, Freiburg, 79104, Germany). Naunyn-Schmiedeberg's Archives of Pharmacology, 366(6), 501-512 (English) 2002. CODEN: NSAPCC. ISSN: 0028-1298. Publisher: Springer-Verlag.

AB A review. Several bacterial protein toxins, including Clostridium botulinum C2 toxin, Clostridum perfringens iota toxin, Clostridium difficile ADP-ribosyltransferase, and the Bacillus-produced vegetative insecticidal proteins, target the cytoskeleton by ADP-ribosylation of actin. All these toxins are binary in structure and consist of an enzyme component, possessing ADP-ribosyltransferase activity and a separated binding and translocation component, which is involved in the delivery of the enzyme component into the cell. The toxins are not only important virulence factors but also cell biol. tools to study the function of the actin cytoskeleton. Moreover, the binary toxins turned out to be effective transporter systems for the delivery of specific **fusion** toxins (e.g., Rho-ADP-ribosylating C3 exoenzyme) into cells. The present review describes the biol. functions of the toxins, focuses on recent studies on the uptake and delivery mechanism and discusses the usage as a drug delivery system.

L10 ANSWER 15 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2001117003 EMBASE Clostridium perfringens iota-toxin: Mapping of receptor binding and Ia docking domains on Ib. Marvaud J.-C.; Smith T.; Hale M.L.; Popoff M.R.; Smith L.A.; Stiles B.G.. J.-C. Marvaud, Toxinology Division, USAMRIID, Fort Detrick, MD 21702-5011, United States. bradley.stiles@amedd.army.mil. Infection and Immunity 69/4 (2435-2441) 2001.
Refs: 36.
ISSN: 0019-9567. CODEN: INFIBR. Pub. Country: United States. Language: English. Summary Language: English.

AB Clostridium perfringens iota-toxin is a binary toxin consisting of iota a (Ia), an ADP-ribosyltransferase that modifies actin, and iota b (Ib), which binds to a cell surface protein and translocates Ia into a target cell. **Fusion** proteins of recombinant Ib and truncated variants were tested for binding to Vero cells and docking with Ia via fluorescence-activated cytometry and cytotoxicity experiments. C-terminal residues (656 to 665) of Ib were critical for cell surface binding, and truncated Ib variants containing ≥200 amino acids of the C terminus were effective Ib competitors and prevented iota cytotoxicity. The N-terminal domain (residues 1 to 106) of Ib was important for Ia docking, yet this region was not an effective competitor of iota cytotoxicity. Further studies showed that Ib lacking just the N-terminal 27 residues did not facilitate Ia entry into a target cell and subsequent cytotoxicity. Five monoclonal antibodies against Ib were also tested with each truncated Ib variant for epitope and structural mapping by surface plasmon resonance and an enzyme-linked immunosorbent assay. Each antibody bound to a linear epitope within the N terminus (residues 28 to 66) or the C terminus (residues 632 to 655). Antibodies that target the C terminus neutralized in vitro cytotoxicity and delayed the lethal effects of iota-toxin in mice.

L10 ANSWER 16 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2001183875 EMBASE Regulation by Rho family GTPases of IL-1 receptor induced signaling: C3-like chimeric toxin and Clostridium difficile toxin B inhibit signaling pathways involved in IL-2 gene expression. Dreikhausen

U.; Varga G.; Hofmann F.; Barth H.; Aktories K.; Resch K.; Szamel M.. M. Szamel, Department of Immunopharmacology, Institute of Pharmacology, Medical School Hannover, D-30623 Hannover, Germany. Szamel.Marta@MH-Hannover.de. European Journal of Immunology 31/5 (1610-1619) 2001. Refs: 36.
ISSN: 0014-2980. CODEN: EJIMAF. Pub. Country: Germany. Language: English. Summary Language: English.

AB In this study the participation of Rho family GTPases in the regulation of IL-1-activated protein kinase cascades controlling IL-2 synthesis was investigated in murine EL-4 thymoma cells. The recombinant C3-like chimeric toxin, which consists of the C3 toxin of Clostridium limosum and the N-terminal part of Clostridium botulinum C2 toxin (C2IN-C3) interacting with the C2II binding subunit to facilitate uptake into cells, and selectively inactivates Rho A by ADP-ribosylation, prevented IL-1-stimulated activation of Jun-NH(2)-terminal-kinases (JNK) and p38 mitogen-activated-protein kinases (MAPK). UDP-monoglucosylation and concomitant inactivation of Rho A and of Rac-2 by Clostridium difficile toxin B also inhibited IL-1-induced activation of JNK and p38 MAPK, but additionally inhibited activation of the extracellular-regulated-kinase pathway and DNA binding of the transcription factor NFκB. Accordingly, pre-treatment of cells with C2IN-C3 **fusion** toxin only decreased IL-1-stimulated IL-2 synthesis by 50%, while in C. difficile toxin B-treated cells IL-1-induced IL-2 secretion was reduced by 90%. These results imply that together with Rho A an additional member of the Rho family G proteins, i.e. Rac-2, is critically involved as an upstream regulator in IL-1-induced activation of different MAPK, stress-activated protein kinases, and in NFκB activation controlling IL-2 gene expression in response to IL-1, acting in close proximity to the IL-1 receptor complex.

L10 ANSWER 17 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN
2001:701138 Document No. 136:396575 Construction of **fusion** gene of Clostridium perfringens of α-toxin and β-toxin. Xu, Chongbo; Zhao, Baohua; Wei, Guangsen; Jiang, Yuwen; Wang, Zhuo (The Military Veterinary Institute, Quartermaster University of PLA, Changchun, 130062, Peop. Rep. China). Zhongguo Shouyi Xuebao, 21(4), 341-343 (Chinese) 2001. CODEN: ZSXUF5. ISSN: 1005-4545. Publisher: Zhongguo Shouyi Xuebao Bianjibu.

AB The construction of **fusion** gene of α-toxin and β-toxin of Clostridium perfringens was studied. The β-toxin gene was amplified from plasmid pXETB2 containing Clostridium perfringens β-toxin gene by polymerase chain reaction (PCR), PCR products were cleaved with restriction endonucleases Nco I and BamH I and recovered. The 3' terminus of β-toxin gene was genetically fused to the 5' terminus of α-toxin gene of Clostridium perfringens. The recombinant plasmid pXCPAB1 was characterized by restriction endonuclease anal. and nucleotide sequencing. The results showed that the recombinant plasmid comprising the α-β **fusion** gene. By transformation of BL21(DE3), the recombinant strain BL21(DE3)(pXCPAB1) was obtained. The recombinant strain was proved to be able to produce α-β toxin **fusion** protein by ELISA and SDS-PAGE.

L10 ANSWER 18 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN
2000:742256 Document No. 133:295361 Clostridium difficile recombinant toxin A repeating units as a carrier protein for conjugate vaccines. Wilkins, Tracy D.; Lylerly, David M.; Moncrief, J. Scott; Pavliakova, Danka; Scheerson, Rachel; Robbins, John B. (Techlab, Inc., USA; United States Dept. of Health and Human Services). PCT Int. Appl. WO 2000061761 A2 20001019, 45 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE,

SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US9523 20000410. PRIORITY: US 1999-PV128686 19990409; US 2000-PV186201 20000301.

AB The present invention provides for immunogenic compns. and their methods of use as vaccines and their method of preparation These immunogenic compns. comprise a recombinant protein of toxin A of Clostridium difficile conjugated to a polysaccharide of a microbial pathogen. The immunogenic compns. may include only a nontoxic truncated portion of toxin A, particularly the repeating units (rARU), that is conjugated to a microbial pathogen polysaccharide. The yields of these polysaccharide-protein conjugates can be significantly increased by prior treatment of rARU with succinic anhydride. Such compns. are effective in eliciting T-cell dependent and antibody responses, and immune responses to pneumococcal type 14, Escherichia coli K1, and Shigella flexneri type 2a polysaccharides in mice are demonstrated. All conjugates elicited high levels of serum IgG both to the polysaccharides and to CDTA. These compns. are therefore effective as vaccines for humans, particularly children, and animals in affording protection against one or more microbial pathogens.

L10 ANSWER 19 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN

2000:381824 Document No. 133:22430 Conjugate for enrichment in neural cells. Loehr, Achim; Schwab, Manfred (Deutsches Krebsforschungszentrum Stiftung des Oeffentlichen Rechts, Germany). Ger. Offen. DE 19856052 A1 20000608, 12 pp. (German). CODEN: GWXXBX. APPLICATION: DE 1998-19856052 19981204.

AB An active substance is introduced specifically into neurons by conjugating it with the heavy chain of a **Clostridium toxin** in the absence of the toxic light chain. The heavy chain and its fragments, modifications, and **fusion** proteins mediate neuron-specific binding, uptake, and release of the conjugate into the cytoplasm, but are nontoxic. The active agent is coupled to an SH group on the heavy chain through a linker via an SS group; alternatively, if the active agent is a nucleic acid, it is bound via H bonds to an oligonucleotide coupled to the heavy chain, or via ionic bonds to a peptide or protein recombinantly fused to the heavy chain. Use of the heavy chain is facilitated by modifying it to mimic the structure of the native toxin; this is done by substituting other, nontoxic proteins for the light chain and binding them to the heavy chain via SS groups. The conjugates can be used for diagnosis and/or therapy of neuronal diseases, e.g. neuroblastoma. Thus, the gene for tetanus toxin heavy chain was cloned, fused with the gene for HMG-1 (high-mobility-group) protein (which nonspecifically binds DNA), and expressed in Escherichia coli.

L10 ANSWER 20 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2000212107 EMBASE Measurement of exocytosis by amperometry in adrenal chromaffin cells: Effects of clostridial neurotoxins and activation of protein kinase C on **fusion** pore kinetics. Graham M.E.; Fisher R.J.; Burgoyne R.D.. R.D. Burgoyne, Physiological Laboratory, University of Liverpool, Crown Street, Liverpool L69 3BX, United Kingdom. burgoyne@liverpool.ac.uk. Biochimie 82/5 (469-479) 2000.
Refs: 54.
ISSN: 0300-9084. CODEN: BICMBE. Pub. Country: France. Language: English. Summary Language: English.

AB We have used carbon-fibre amperometry to examine the kinetics of individual secretory granule **fusion**/release events in bovine adrenal chromaffin cells. Transfection with plasmids encoding the light chains of botulinum neurotoxins (BoNTs) was used to investigate the effects of cleavage of syntaxin or SNAP-25 on exocytosis. Expression of BoNT/C1 or BoNT/E inhibited the extent of exocytosis that was evoked by application of digitonin/Ca2+ to permeabilise and stimulate single chromaffin cells. Following neurotoxin expression, the residual release events were no different from those of control cells in their magnitude and kinetics from analysis of the amperometric spikes. In contrast, activation of protein kinase C (PKC) resulted in a modification of the kinetics of single granule release events. Following phorbol ester

treatment, the amperometric spikes showed a significant decrease in their total charge due to a decrease in their mean half-width with increases in the rate of the initial rise and also the fall to baseline of the spikes. These changes were prevented by pre-treatment with the PKC inhibitor bisindolylmaleimide. These results suggest that PKC regulates the rate of **fusion** pore expansion and also subsequent pore closure or granule retrieval. A PKC-mediated regulation of kiss-and-run **fusion** may, therefore, control the extent of catecholamine release from single secretory granules. The experimental approach used here may provide further information on the protein constituents and regulation of the **fusion** pore machinery. (C) 2000 Societe francaise de biochimie et biologie moleculaire / Editions scientifiques et medicales Elsevier SAS.

L10 ANSWER 21 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2000435436 EMBASE Clostridial neurotoxin-insensitive vesicular SNAREs in exocytosis and endocytosis. Martinez-Arca S.; Alberts P.; Galli T.. T. Galli, Group of Membrane Traffic, Neuronal Plasticity, Institut Curie, 26 Rue d'Ulm, 75231 Paris Cedex 05, France. thierry.galli@curie.fr. Biology of the Cell 92/6 (449-453) 2000.
Refs: 42.
ISSN: 0248-4900. CODEN: BCELDF. Pub. Country: France. Language: English.

L10 ANSWER 22 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2000384239 EMBASE Evidence for a vesicle-mediated maintenance of store-operated calcium channels in a human embryonic kidney cell line. Alderton J.M.; Ahmed S.A.; Smith L.A.; Steinhardt R.A.. Dr. R.A. Steinhardt, Department of Molecular/Cell Biology, University of California, 391 LSA 3200, Berkeley, CA 94720-3200, United States. rsteinha@socrates.berkeley.edu. Cell Calcium 28/3 (161-169) 2000.
Refs: 46.
ISSN: 0143-4160. CODEN: CECADV. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Direct microinjection of the clostridial neurotoxins botulinum neurotoxin A light chain or tetanus neurotoxin into cells of a human embryonic kidney cell line significantly reduced calcium entry after depletion of internal calcium stores by cyclopiazonic acid, a reversible inhibitor of the sarcoplasmic-endoplasmic reticular calcium-ATPases. Botulinum neurotoxin A light chain specifically hydrolyzes a synaptosomal-associated protein of 25 kilodaltons (SNAP-25), and tetanus neurotoxin specifically hydrolyzes synaptobrevin-2 (vesicle-associated membrane protein 2, VAMP-2) and cellubrevin (vesicle-associated membrane protein 3, VAMP-3). Since these substrate proteins are required for vesicle docking and **fusion**, inhibition of store-operated calcium entry by botulinum neurotoxin A light chain and tetanus neurotoxin supports a model in which vesicle **fusion** is a prerequisite for activation of store-operated calcium entry. Brefeldin A, a fungal metabolite that interferes with vesicle traffic, partially reduced calcium entry following store depletion. The size of the reserve pool of vesicles or parallel vesicle recycling pathways employing brefeldin A-sensitive and brefeldin A-insensitive ADP-ribosylation factors may explain the failure of brefeldin A to completely inhibit store-operated calcium entry. (C) 2000 Harcourt Publishers Ltd.

L10 ANSWER 23 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN

1999:425464 Document No. 131:57767 Clostridium difficile toxins as mucosal adjuvants. Thomas, William D., Jr.; Monath, Thomas P.; Zhang, Zhenxi; Torres-Lopez, Francisco Javier; Lei, Wende; Lyerly, David M.; Moncrief, James S. (Oravax, Inc., USA). U.S. US 5919463 A 19990706, 19 pp., Cont.-in-part of U.S. Ser. No. 499,384, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1995-543708 19951016. PRIORITY: US 1995-499384 19950707.

AB The invention features methods and compns. for inducing protective and/or therapeutic immune responses to an antigen in a mammal. In these methods,

an antigen is administered to the mammal with a cytotoxin or enterotoxin of a Clostridium, or a fragment or derivative thereof having mucosal adjuvant activity. Thus, Clostridium difficile toxin A and B and toxin A **fusion** protein were used as mucosal adjuvant for immunization with ovalbumin, keyhole limpet hemocyanin, and Helicobacter pylori urease. The effectiveness of the adjuvants administered through rectal and vaginal immunization routes were demonstrated.

L10 ANSWER 24 OF 53 EMBASE
on STN

1999324673 EMBASE SNARE proteins regulate H+-ATPase redistribution to the apical membrane in rat renal inner medullary collecting duct cells.
Banerjee A.; Shih T.; Alexander E.A.; Schwartz J.H.. J.H. Schwartz, Evans 401, One Boston Medical Center Place, Boston, MA 02118-2908, United States. jhsch@bu.edu. Journal of Biological Chemistry 274/37 (26518-26522) 10 Sep 1999.
Refs: 31.
ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.

AB The interaction of soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins provides the necessary steps for vesicle docking **fusion.** In inner medullary collecting duct (IMCD) cells, acid secretion is regulated in part by exocytotic insertion and endocytotic retrieval of an H+-ATPase to and from the apical membrane. We previously suggested a role for SNARE proteins in exocytotic insertion of proton pumps in IMCD cells. The purpose of the present study was to determine whether SNARE proteins are associated with the 31-kDa subunit of H+-ATPase in IMCD cells during exocytosis and to determine the effects of clostridial toxins on SNARE-mediated trafficking of H+-ATPase. Cell acidification induced a marked increment of H+-ATPase in the apical membrane. However, pretreating cells with clostridial toxins blocked the cellular translocation of the 31-kDa subunit. Immunoprecipitation of IMCD cell homogenate, using antibodies against either the 31-kDa subunit of H+-ATPase or vesicle-associated membrane protein-2, co-immunoprecipitated N-ethylmaleimide-sensitive factor, α-soluble NSF attachment protein (α-SNAP), synaptosome-associated protein- 23, syntaxin, and vesicle-associated membrane protein-2. Pretreatment with clostridial toxin resulted in reduced co-immunoprecipitation of H+-ATPase and syntaxin. These experiments document, for the first time, a putative docking **fusion** complex in IMCD cells and a physical association of the H+-ATPase with the complex. The sensitivity to the action of clostridial toxin indicates the docking-**fusion** complex is a part of the exocytotic mechanism of the proton pump.

L10 ANSWER 25 OF 53 CAPLUS

1999:291170 Document No. 131:72427 Immunogenicity of a Salmonella typhimurium aroA aroD vaccine expressing a nontoxic domain of Clostridium difficile toxin A. Ward, Stephen J.; Douce, Gill; Figueiredo, Dayse; Dougan, Gordon; Wren, Brendan W. (Microbial Pathogenicity Research Group, Department of Microbiology, St. Bartholomew's and the Royal London School of Medicine and Dentistry, London, EC1A 7BE, UK). Infection and Immunity, 67(5), 2145-2152 (English) 1999. CODEN: INFIBR. ISSN: 0019-9567. Publisher: American Society for Microbiology.

AB The C-terminal repeat domain of Clostridium difficile toxin A harbors toxin-neutralizing epitopes and is considered to be a candidate component of a vaccine against C. difficile-associated disease (CDAD). Fourteen of the 38 C-terminal toxin A repeats (14CDTA) were cloned into pTECH-1 in frame with the immunogenic fragment C of tetanus toxin (TETC) to generate plasmid p56TETC. Expression of the TETC-14CDTA **fusion** protein was driven from the anaerobically inducible nirB promoter within attenuated Salmonella typhimurium BRD509 (aroA aroD). The TETC-14CDTA **fusion** protein was purified and shown to bind to known toxin A receptors found on the surface of rabbit erythrocytes. Intranasal (i.n.) and intragastric (i.g.) immunization with 107 and 1010 CFU, resp., of BRD509(p56TETC) generated significant anti-toxin A serum responses after a

single dose. Antibody titers were elevated following a boosting dose with either live vaccine or a s.c. injection of 0.5 μg of purified 14CDTA protein. Importantly, serum from mice immunized with BRD509(p56TETC) neutralized toxin A cytotoxicity. Both i.n. and i.g. immunizations also generated toxin A-specific IgA on the pulmonary and intestinal mucosa, resp. Intranasal vaccination induced consistently higher serum and mucosal anti-toxin A antibody responses. Significant anti-tetanus toxoid serum and mucosal antibodies were also generated by both immunization routes. The availability of live attenuated Salmonella typhi for human use may allow the development of a multivalent mucosal vaccine against CDAD, tetanus, and typhoid.

L10 ANSWER 26 OF 53 EMBASE
on STN
1999099246 EMBASE Structure-function relationship of clostridial neurotoxins.
Li L.; Singh B.R.. L. Li, Department Chemistry Biochemistry, University Massachusetts Dartmouth, Dartmouth, MA 02747, United States. Journal of Toxicology - Toxin Reviews 18/1 (95-112) 1999.
Refs: 83.
ISSN: 0731-3837. CODEN: JTTRD. Pub. Country: United States. Language: English. Summary Language: English.
AB Botulinum neurotoxins are a unique group of metalloproteases which catalyze single site cleavage of specific proteins involved in the docking and **fusion** of synaptic vesicles with plasma membrane for neurotransmitter release. Seven serotypes of botulinum neurotoxins share a common molecular mode of action, with remarkable difference in their primary amino acid sequences and protein substrates in neuronal cells. The neurotoxins are large water soluble proteins (150 kDa) with distinct domains associated with different biochemical functions during the toxicogenesis process. In this review, we have focused on the description of the role of specific protein segments in the binding, translocation and endopeptidase activity of the toxin molecules.

L10 ANSWER 27 OF 53 CAPLUS
1999:27954 Document No. 130:77075 Targetting and uptake of DNA by animal cells by receptor-mediated endocytosis using **fusion** protein of toxins and DNA-binding proteins. Grandi, Guido (Chiron S.P.A., Italy). PCT Int. Appl. WO 9859065 A1 19981230, 85 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1998-IB1005 19980618. PRIORITY: GB 1997-13122 19970620.
AB A method of using receptor-mediated endocytosis to increase the efficiency of DNA uptake by eukaryotic cells is described. The method uses **fusion** proteins of receptor-binding domains of toxins, therefore lacking the domains necessary for toxic activity, and DNA-binding domains. These **fusion** proteins are taken up by the receptor for the toxin and the DNA it is bound to is incorporated into the endosome. When the endosome is internalized, the complex is released and the protein stripped from the DNA leaving it free to become part of the host cell genome. A **fusion** protein of the heat-labile enterotoxin of Escherichia coli and the histone H1-like protein of Bordetella pertussis was prepared by expression of the cloned gene. The protein was shown to retain DNA binding activity. Similarly, a **fusion** protein of diphtheria toxin and GAL4 was shown to have DNA binding and to retain the normal binding of the toxin to Vero cells. The **fusion** protein was also rapidly internalized by Vero cells.

L10 ANSWER 28 OF 53 CAPLUS
1999:27942 Document No. 130:94471 Immunogenic fragments of toxin A of Clostridium difficile. Ward, Stephen John; Wren, Brendan William; Dougan, Gordon; Douce, Gill (Queen Mary & Westfield College, UK; Imperial College of Science, Technology & Medicine). PCT Int. Appl. WO 9859053 A1 19981230, 82 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,

MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-GB1805 19980619. PRIORITY: GB 1997-13146 19970620; GB 1998-321 19980107.

AB The present invention relates to the 14-repeat C-terminal region of Clostridium difficile toxin A that is effective in generating anti-toxin A antibodies and thereby has the ability to act as a vaccine against C. difficile infection. This 14-repeat fragment (encoded by nucleotides 7159-8118 of the toxin A gene from C. difficile strain VPI 10463) is superior to the whole C-terminal repeat region and other subfragments in generating immunity to C. difficile.

L10 ANSWER 29 OF 53 MEDLINE on STN DUPLICATE 1

1998328523. PubMed ID: 9665586. SNAP-25 and syntaxin, but not synaptobrevin 2, cooperate in the regulated release of nerve growth factor. Blochl A. (Molekulare Neurobiochemie, Ruhr-Universitat Bochum, Germany.) Neuroreport, (1998 Jun 1) 9 (8) 1701-5. Journal code: 9100935. ISSN: 0959-4965. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Investigating the release mechanism of nerve growth factor (NGF) the possible cooperation of SNAREs (target (t)- and vesicular (v)- soluble NSF attachment protein receptors) which form a **fusion** core during docking of synaptic vesicles at the plasma membrane was examined. Cleavage of those proteins by **clostridium toxins** shows that SNAP-25 (synaptic vesicle associated protein) and syntaxin are involved but not synaptobrevin 2. Alpha-latrotoxin, which effects the release of neurotransmitters by acting on the **fusion** core, also induces Ca2+-independent NGF release. Taken together, the results indicate the formation of a **fusion** core during regulated NGF release and implicate the existence of NGF vesicles.

L10 ANSWER 30 OF 53 EMBASE on STN

1998071491 EMBASE Chimeric clostridial cytotoxins: Identification of the N-terminal region involved in protein substrate recognition. Hofmann F.; Busch C.; Aktories K.. K. Aktories, Inst. fur Pharmakologie/Toxikologie, Albert-Ludwigs-Universitat Freiburg, Hermann-Herder-Str. 5, 79104 Freiburg, Germany. aktories@uni-freiburg.de. Infection and Immunity 66/3 (1076-1081) 1998.
Refs: 29.
ISSN: 0019-9567. CODEN: INFIBR. Pub. Country: United States. Language: English. Summary Language: English.

AB Clostridium sordellii lethal toxin is a member of the family of large clostridial cytotoxins that glucosylate small GTPases. In contrast to Clostridium difficile toxins A and B, which exclusively modify Rho subfamily proteins, C. sordellii lethal toxin also glucosylates Ras subfamily proteins. By deletion analysis and construction of chimeric **fusion** proteins of C. sordellii lethal toxin and C. difficile toxin B, we localized the enzyme activity of the lethal toxin to the N terminus of the holotoxin and identified the region involved in protein substrate specificity. The toxin fragment of the N-terminal 546 amino acid residues of C. sordellii lethal toxin glucosylated Rho and Ras subfamily proteins, as the holotoxin did. Deletion of a further 30 amino acid residues from the C terminus of this active fragment drastically reduced glucotransferase activity and blocked glucohydrolase activity. Exchange of amino acid residues 364 through 516 of lethal toxin for those in the active toxin B fragment (1 to 546) allowed glucosylation of Ras subfamily proteins. In contrast, the chimera with amino acids 1 to 364 from toxin B, 365 to 468 from lethal toxin, and 469 to 546 from toxin B exhibited markedly reduced modification of Ras subfamily proteins, whereas modification of Rac and Cdc42 was hardly changed. The data indicate that the region of amino acid residues 364 through 516 primarily defines the substrate specificity of C. sordellii lethal toxin.

L10 ANSWER 31 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

1998163190 EMBASE Widespread association of annexin II with intracellular membrane systems and involvement of annexin II in granule-granule **fusion** in addition to granule-plasma membrane **fusion** in anterior pituitary cells. Senda T.; Yu W.; Okabe T.; Sugimoto N.; Matsuda M.. T. Senda, Department of Anatomy I, Nagoya University School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466, Japan. Histochemical Journal 30/5 (331-338) 1998.
Refs: 41.
ISSN: 0018-2214. CODEN: HISJAE. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB The subcellular localization in anterior pituitary secretory cells of annexin II, one of the Ca2+-dependent phospholipid-binding proteins, was examined by immunohistochemistry and immunoelectron microscopy. Annexin II was associated with the plasma membrane, the membranes of secretory granules and cytoplasmic organelles, such as rough endoplasmic reticulum, mitochondria and vesicles, and with the nuclear envelope. Annexin II was frequently detected at the contact sites of secretory granules with other granules and with the plasma membrane. The anterior pituitary and adrenal medulla were treated with Clostridium perfringens enterotoxin, which induces Ca2+ influx, and examined under an electron microscope. The anterior pituitary cells showed multigranular exocytosis, i.e. multiple **fusions** of secretory granules with each other and with the plasma membrane, but adrenal chromaffin cells, which lack annexin II on the granule membranes, never showed granule- granule **fusion** and only single granule exocytosis. From these results, we conclude that, in anterior pituitary secretory cells, annexin II is involved in granule-granule **fusion** in addition to granule-plasma membrane **fusion.**

L10 ANSWER 32 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

1998016136 EMBASE Identification and characterization of sporulation-dependent promoters upstream of the enterotoxin gene (cpe) of Clostridium perfringens. Zhao Y.; Melville S.B.. S.B. Melville, Dept. of Microbiology and Immunology, University of Tennessee, 858 Madison Ave., Memphis, TN 38163, United States. sbmelville@utmem1.utmem.edu. Journal of Bacteriology 180/1 (136-142) 1998.
Refs: 30.
ISSN: 0021-9193. CODEN: JOBAAY. Pub. Country: United States. Language: English. Summary Language: English.

AB Three promoter sites (P1, P2, and P3) responsible for the sporulation-associated synthesis of Clostridium perfringens enterotoxin, a common cause of food poisoning in humans and animals, were identified. Nested and internal deletions of the cpe promoter region were made to narrow down the location of promoter elements. To measure the effects of the deletions on the expression of cpe, translational **fusions** containing the promoter deletions were made with the gusA gene of Escherichia coli, which codes for β-glucuronidase; E. coli-C. perfringens shuttle vectors carrying the **fusions** were introduced into C. perfringens by electroporation. In addition, in vitro transcription assays were performed with the cpe promoter region as the DNA template for extracts made from sporulating cells. DNA sequences upstream of P1 were similar to consensus SigK-dependent promoters, while P2 and P3 were similar to consensus SigE-dependent promoters. SigE and SigK are sporulation-associated sigma factors known to be active in the mother cell compartment of sporulating cells of Bacillus subtilis, the same compartment in which enterotoxin is synthesized in C. perfringens.

L10 ANSWER 33 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN

1997:187122 Document No. 126:185040 Clostridium difficile toxins as mucosal adjuvants. Thomas, William D., Jr.; Monath, Thomas P.; Zhang, Zhenxi; Torres-Lopez, Francisco Javier; Lei, Wende; Lyerly, David M.; Moncrief, James S. (Oravax, Inc., USA). PCT Int. Appl. WO 9702836 A1 19970130, 42

pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US11142 19960701. PRIORITY: US 1995-499384 19950707; US 1995-543708 19951016.

AB The invention features methods and compns. for inducing protective and/or therapeutic immune responses to an antigen in a mammal. In these methods, an antigen is administered to the mammal with a toxin of a Clostridium (e.g., C. difficile), or a fragment or derivative thereof having adjuvant activity. Demonstrated were adjuvant activities of C. difficile toxin A when co-administered with ovalbumin, keyhole limpet hemocyanin, and Helicobacter pylori urease. Also **fusion** protein containing glutathione S-transferase and repeats which make up the carbohydrate binding domain of toxin A was prepared and used as immune adjuvant.

L10 ANSWER 34 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN

1997:184687 Document No. 126:170388 Intranasal vaccination against gastrointestinal disease. Thomas, William D., Jr.; Monath, Thomas P.; Torres-Lopez, Francisco; Zhang, Zhenxi; Lei, Wende; Lyerly, David M.; Moncrief, James S. (Oravax, Inc., USA). PCT Int. Appl. WO 9702835 A1 19970130, 52 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US10987 19960626. PRIORITY: US 1995-499229 19950707.

AB The invention features intranasal immunization methods for inducing immune responses in distal mucosal sites, e.g., the gastrointestinal or genitourinary tracts. The methods of the invention may be used to induce protective and/or therapeutic immune responses against pathogens (e.g., bacteria of the genus Clostridium, e.g., C. difficile) which infect these distal sites. Also included in the invention are vaccination methods in which combinations of mucosal (e.g., oral or intranasal) and parenteral (e.g., s.c. or i.p.) routes of administration are used. Vaccines containing C. difficile toxin A or B or toxin A **fusion** protein were prepared for immunization of hamsters and mice.

L10 ANSWER 35 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN

1997:776762 Document No. 127:355944 **Fusion** proteins of immunopotentiating activity and **fusions** of bacterial toxins with specific cell receptors. Lowenadler, Bjorn; Lycke, Nils (Lowenadler, Bjorn, Swed.; Lycke, Nils). Can. Pat. Appl. CA 2168914 AA 19970807, 28 pp. (English). CODEN: CPXXEB. APPLICATION: CA 1996-2168914 19960206.

AB The claims include a DNA-sequence comprising a first sequence coding for a native or mutant subunit of a bacterial toxin that confers enzymic ADP-ribosylating activity, and a second sequence coding for a peptide such that the resulting **fusion** protein is in possession of water solubility and capability of targeting the **fusion** protein to a specific cell receptor different from receptors binding to the native toxin, thereby mediating intracellular uptake of at least said subunit. Also claimed are **fusion** proteins coded for by such DNA-sequence; compns. for use in improving immune functions; and recombinant expression vectors and transformed bacterial cells containing such DNA-sequence. The bacterial toxins may include cholera toxin, Escherichia coli heat-labile enterotoxin, and toxins of Pertussis, Clostridium, Shigella, and Pseudomonas. The cell receptor may be for lymphocytes and monocytes, for Ig or Fc, or for antigen presentation.

L10 ANSWER 36 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 2

97188301 EMBASE Document No.: 1997188301. Protective immunity against Clostridium difficile toxin A induced by oral immunization with a live,

attenuated Vibrio cholerae vector strain. Ryan E.T.; Butterton J.R.; Smith R.N.; Carroll P.A.; Crean T.I.; Calderwood S.B.. S.B. Calderwood, Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA 02114, United States. Infection and Immunity 65/7 (2941-2949) 1997.
Refs: 63.
ISSN: 0019-9567. CODEN: INFIBR. Pub. Country: United States. Language: English. Summary Language: English.

AB Clostridium difficile causes pseudomembranous colitis through the action of Rho-modifying proteins, toxins A and B. Antibodies directed against C. difficile toxin A prevent or limit C. difficile-induced colitis. We engineered plasmid pETR14, containing the hlyB and hlyD genes of the Escherichia coli hemolysin operon, to express a **fusion** protein containing 720 amino acid residues from the nontoxic, receptor-binding, carboxy terminus of C. difficile toxin A and the secretion signal of E. coli hemolysin A. We introduced pETR14 into Vibrio cholerae and found that the toxin A-HlyA **fusion** protein was secreted by a number of V. cholerae strains and recognized by both monoclonal and polyclonal anti-C. difficile toxin A antibodies. We introduced pETR14 into an attenuated V. cholerae strain, 0395-NT, and inoculated rabbits orally with this construct. Colonization studies disclosed that the V. cholerae vector containing pETR14 was recoverable from rabbit ilea up to 5 days after oral inoculation. Vaccination produced significant systemic anti-C. difficile toxin A immunoglobulin G and anti-V. cholerae vibriocidal antibody responses. Vaccination also produced significant protection against toxin A in an ileal loop challenge assay, as assessed by determination of both fluid secretion and histological changes. These results suggest that the hemolysin system of E. coli can be used successfully in V. cholerae vector strains to effect secretion of large heterologous antigens and that a V. cholerae vector strain secreting a nontoxic, immunogenic portion of C. difficile toxin A fused to the secretion signal of E. coli HlyA induces protective systemic and mucosal immunity against this toxin.

L10 ANSWER 37 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN
1997:139125 Document No. 126:247459 Positive regulation of Clostridium difficile toxins. Moncrief, J. Scott; Barroso, Lisa A.; Wilkins, Tracy D. (TechLab, Inc., Blacksburg, VA, 24061-0346, USA). Infection and Immunity, 65(3), 1105-1108 (English) 1997. CODEN: INFIBR. ISSN: 0019-9567. Publisher: American Society for Microbiology.

AB The toxigenic element of Clostridium difficile VPI 10463 contains a small open reading frame (ORF) immediately upstream of the toxin B gene. The deduced amino acid sequence of the ORF, which was designated txeR, encodes a 22-kDa protein which contains a helix-turn-helix motif with sequence identity to DNA binding regulatory proteins. A DNA fragment containing the C. difficile toxin A repeating units (ARU) was used as a reporter gene to determine if txeR regulates expression from the toxin A and toxin B promoters in Escherichia coli. To test the affect of txeR on expression, the ARU gene fragment was fused in frame with the toxin promoters. The **fusions** expressed a 104-kDa protein that contained the epitopes for monoclonal antibody PCG-4, which was used to measure levels of recombinant ARU by ELISA. When txeR was expressed in trans with the toxin B promoter-ARU **fusion** contained on sep. low-copy-number plasmid, expression of ARU increased over 800-fold. Furthermore, when the toxin A promoter fused to ARU was tested, expression increased over 500-fold with txeR supplied in trans. These results suggest that TxeR is a pos. regulator that activates expression of the C. difficile toxins.

L10 ANSWER 38 OF 53 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
1998080383 EMBASE H+ secretion is inhibited by clostridial toxins in an inner medullary collecting duct cell line. Alexander E.A.; Shih T.; Schwartz J.H.. E.A. Alexander, Renal Section, Evans 401, One Boston Medical Place, Boston, MA 02118-2908, United States. American Journal of Physiology - Renal Physiology 273/6 42-6 (F1054-F1057) 1997.
Refs: 17.
ISSN: 0363-6127. CODEN: AJPPFK. Pub. Country: United States. Language:

English. Summary Language: English.

AB Renal epithelial cell H+ secretion is an exocytic-endocytic phenomenon. In the inner medullary collecting duct (IMCD) cell line, which we have utilized as a model of renal epithelial cell acid secretion, we found previously that acidification increased exocytosis and alkalinization increased endocytosis. It is likely, therefore, that the rate of proton secretion is regulated by the membrane insertion and retrieval of proton pumps. There is abundant evidence from studies in the nerve terminal and the chromaffin cell that vesicle docking, membrane **fusion**, and discharge of vesicular contents (exocytosis) involve a series of interactions among so- called trafficking proteins. The clostridial toxins, botulinum and tetanus, are proteases that specifically inactivate some of these proteins. In these experiments we demonstrated, by immunoblot and immunoprecipitation, the presence in this IMCD cell line of the specific protein targets of these toxins, synaptobrevin/vesicle-associated membrane proteins (VAMP), syntaxin, and synaptosomal-associated protein-25 (SNAP-25). Furthermore, we showed that these toxins markedly inhibit the capacity of these cells to realkalinize after an acid load. Thus these data provide new insight into the mechanism for H+ secretion in the IMCD.

L10 ANSWER 39 OF 53 EMBASE
on STN

97016472 EMBASE Document No.: 1997016472. Bacterial protein toxins and cell vesicle trafficking. Montecucco C.; Papini E.; Schiavo G.. C. Montecucco, Centro CNR Biomembrane, Dipartimento di Scienze Biomediche, Universita di Padova, Via Trieste 75, I-35121 Padova, Italy. toxin@cribi1.bio.unipd.it.
Experientia 52/12 (1026-1032) 1996.
Refs: 91.
ISSN: 0014-4754. CODEN: EXPEAM. Pub. Country: Switzerland. Language: English. Summary Language: English.

AB A group of bacterial protein toxins interfere with vesicular trafficking inside cells. Clostridial neurotoxins affect mainly the highly regulated **fusion** of neurotransmitter- and hormone-containing vesicles with the plasma membrane. They cleave the three SNARE proteins: VAMP, SNAP-25 and syntaxin, and this selective proteolysis results in a blockade of exocytosis. The Helicobacter pylori cytotoxin is implicated in the pathogenesis of gastroduodenal ulcers. It causes a progressive and extensive vacuolation of cells followed by necrosis, after a cytotoxin-induced alteration of membrane trafficking by late endosomes. Vacuoles originate from this compartment in a rab7-dependent process and swell because they are acidic and accumulate membrane-permeant amines.

L10 ANSWER 40 OF 53 EMBASE
on STN

95362900 EMBASE Document No.: 1995362900. The membrane **fusion** machine and neurotransmitter release. Fesce R.; Valtorta F.; Meldolesi J.. CNR Cellular Molecular Pharmacology, B Ceccarelli Ctrs., Dept. Pharmacol., Univ. of Milan, DIBIT HS Raffaele, via Olgettina 58,20132, Milano, Italy.
Neurochemistry International 28/1 (15-21) 1996.
ISSN: 0197-0186. CODEN: NEUIDS. Pub. Country: United Kingdom. Language: English.

L10 ANSWER 41 OF 53 EMBASE
on STN

95307952 EMBASE Document No.: 1995307952. Clostridial neurotoxins compromise the stability of a low energy SNARE complex mediating NSF activation of synaptic vesicle **fusion.** Pellegrini L.L.; O'Connor V.; Lottspeich F.; Betz H.. Abteilung Neurochemie, Max-Planck-Institut Hirnforschung, Deutschordenstrasse 46,D-60528 Frankfurt am Main, Germany.
EMBO Journal 14/19 (4705-4713) 1995.
ISSN: 0261-4189. CODEN: EMJODG. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB A 20S complex composed of the cytosolic **fusion** proteins NSF and SNAP and the synaptosomal SNAP receptors (SNAREs) synaptobrevin, syntaxin

and SNAP-25 is essential for synaptic vesicle exocytosis. Formation of this complex is thought to be regulated by synaptotagmin, the putative calcium sensor of neurotransmitter release. Here we have examined how different inhibitors of neurotransmitter release, e.g. clostridial neurotoxins and a synaptotagmin peptide, affect the properties of the 20S complex. Cleavage of synaptobrevin and SNAP-25 by the neurotoxic clostridial proteases tetanus toxin and botulinum toxin A had no effect on assembly and disassembly of the 20S complex; however, the stability of its SDS-resistant SNARE core was compromised. This SDS-resistant low energy conformation of the SNAREs constitutes the physiological target of NSF, as indicated by its ATP-dependent disassembly in the presence of SNAP and NSF. Synaptotagmin peptides caused inhibition of in vitro binding of this protein to the SNAREs, a result that is inconsistent with synaptotagmin's proposed role as a regulator of SNAP binding. Our data can be reconciled by the idea that NSF and SNAP generate synaptotagmin-containing intermediates in synaptic vesicle **fusion**, which catalyse neurotransmitter release.

L10 ANSWER 42 OF 53 EMBASE
on STN
95164413 EMBASE Document No.: 1995164413. Disassembly of the reconstituted synaptic vesicle membrane **fusion** complex in vitro. Hayashi T.; Yamasaki S.; Nauenburg S.; Binz T.; Niemann H.. Department of Microbiology, Fed Res Ctr Virus Diseases Animals, Paul Ehrlich Strasse 28,D72076 Tubingen, Germany. EMBO Journal 14/10 (2317-2325) 1995.
ISSN: 0261-4189. CODEN: EMJODG. Pub. Country: United Kingdom. Language: English. Summary Language: English.
AB The interaction of the presynaptic membrane proteins SNAP-25 and syntaxin with the synaptic vesicle protein synaptobrevin (VAMP) plays a key role in the regulated exocytosis of neurotransmitters. Clostridial neurotoxins, which proteolyze these polypeptides, are potent inhibitors of neurotransmission. The cytoplasmic domains of the three membrane proteins join into a tight SDS-resistant complex. Here, we show that this reconstituted complex, as well as heterodimers composed of syntaxin and SNAP-25, can be disassembled by the concerted action of the N-ethylmaleimide-sensitive factor, NSF, and the soluble NSF attachment protein, α-SNAP α-SNAP binds to predicted α-helical coiled-coil regions of syntaxin and SNAP-25, shown previously to be engaged in their direct interaction. Synaptobrevin, although incapable of binding α-SNAP individually, induced a third α-SNAP binding site when associated with syntaxin and SNAP-25 into heterotrimers. NSF released prebound α-SNAP from full-length syntaxin but not from a syntaxin derivative truncated at the N-terminus. Disassembly of complexes containing this syntaxin mutant was impaired, indicating a critical role for the N-terminal domain in the α-SNAP/NSF-mediated dissociation process. Complexes containing C-terminally deleted SNAP-25 derivatives, as generated by botulinal toxins type A and E, were dissociated more efficiently. In contrast, the N-terminal fragment generated from synaptobrevin by botulinal toxin type F produced an SDS-sensitive complex that was poorly dissociated.

L10 ANSWER 43 OF 53 EMBASE
on STN
96201691 EMBASE Document No.: 1996201691. Botulinum and tetanus neurotoxins: Emerging tools for the study of membrane **fusion.** Jahn R.; Hanson P.I.; Otto H.; Ahnert-Hilger G.. Department of Pharmacology, Howard Hughes Medical Institute, Yale University Medical School,New Haven, CT 06536, United States. Cold Spring Harbor Symposia on Quantitative Biology 60/- (329-335) 1995.
ISSN: 0091-7451. CODEN: CSHSAZ. Pub. Country: United States. Language: English.

L10 ANSWER 44 OF 53 CAPLUS
1994:291810 Document No. 120:291810 Clostridium difficile toxin B acts on the GTP-binding protein Rho. Just, Ingo; Fritz, Gerhard; Aktories, Klaus;

Giry, Murielle; Popoff, Michel R.; Boquet, Patrice; Hegenbarth, Silke; von Eichel-Streiber, Christoph (Inst. Pharmakol. Toxikol., Univ. Saarlandes, Homburg-Saar, D-66421, Germany). Journal of Biological Chemistry, 269(14), 10706-12 (English) 1994. CODEN: JBCHA3. ISSN: 0021-9258.

AB C. difficile toxin B exhibits cytotoxic activity that is characterized by the disruption of the microfilamental cytoskeleton. Here the authors studied whether the GTP-binding Rho protein, which reportedly participates in the regulation of the actin cytoskeleton, is involved in the toxin action. Toxin B treatment of Chinese hamster ovary cells reveals a time- and concentration-dependent decrease in the ADP-ribosylation of Rho by Clostridium botulinum C3 exoenzyme in the cell lyzate. Disruption of the microfilament system induced by C. botulinum C2 toxin or cytochalasin D does not cause impaired ADP-ribosylation of Rho. Toxin B exhibits its effects on Rho not only in intact cells but also when added to cell lyzates. Besides endogenous Rho, RhoA-glutathione S-transferase (Rho-GST) **fusion** protein added to cell lysate showed decreased ADP-ribosylation after toxin B treatment. Immunoblot anal. reveals identical amts. of Rho-GST and no change in mol. mass after toxin B treatment compared with controls. ADP-ribosylation of Rho-GST purified from toxin B-treated cell lyzate is inhibited, indicating a modification of Rho itself. Finally, transfection of rhoA DNA under the control of a strong promoter into cells protects them from the activity of toxin B. Altogether, the data indicate that C. difficile toxin B acts directly or indirectly on Rho proteins to inhibit ADP-ribosylation and suggest that the cytotoxic effect of toxin B involves Rho.

L10 ANSWER 45 OF 53 EMBASE on STN

94366623 EMBASE Document No.: 1994366623. Expression from the Clostridium perfringens cpe promoter in C. perfringens and Bacillus subtilis. Melville S.B.; Labbe R.; Sonenshein A.L.. Molecular Biology/Microbiology Dept., Tufts University School of Medicine, 136 Harrison Ave.,Boston, MA 02111, United States. Infection and Immunity 62/12 (5550-5558) 1994. ISSN: 0019-9567. CODEN: INFIBR. Pub. Country: United States. Language: English. Summary Language: English.

AB Clostridium perfringens is a source of food poisoning in humans and animals because of production of a potent enterotoxin (CPE). To study the regulation of the cpe gene in C. perfringens, we cloned and sequenced the cpe promoter regions and N-terminal domains from three strains. The cpe promoter region from one strain contained a 45-bp insertion compared with previously published sequences. This insertion was also found in two (of five) other Cpe+ strains, cpe gene expression in C. perfringens was measured by using translational **fusions** of each promoter type to the Escherichia coli gusA gene, which codes for β-glucuronidase. For either promoter type, cpe-gusA expression was undetectable throughout exponential growth but increased dramatically at the beginning of the stationary phase. To measure cpe expression in Bacillus subtilis, cpe-gusA **fusions** were integrated into the B. subtilis chromosome. Both types of promoter exhibited moderate expression during exponential growth; cpe expression increased threefold at the beginning of the stationary phase. Transcriptional start sites were determined by primer extension and in vitro transcription assays. For C. perfringens, both types of promoter gave the same 5' end, 197 bp upstream of the translation start (50 bp downstream of the 45-bp insertion). In B. subtilis, however, the 5' end was internal to the 45-bp insertion, suggesting the use of a different promoter than that utilized by C. perfringens.

L10 ANSWER 46 OF 53 CAPLUS

1994:572667 Document No. 121:172667 Probing the action of Clostridium difficile toxin B in Xenopus laevis oocytes. Just, Ingo; Richter, Hans Peter; Prepens, Ulrike; von Eichel-Streiber, Christoph; Aktories, Klaus (Institut fuer Pharmakologie und Toxikologie, Universitaet des Saarlandes, Homburg/Saar, D-66421, Germany). Journal of Cell Science, 107(6), 1653-9 (English) 1994. CODEN: JNCSAI. ISSN: 0021-9533.

AB Clostridium difficile toxin B and Clostridium botulinum C3 exoenzyme

caused comparable morphol. alteration of CHO cells, which was accompanied by disaggregation of the microfilamental cytoskeleton. The cytotoxic effect of toxin B was correlated with a decrease in C3-catalyzed ADP-ribosylation of the low-mol.-mass GTP-binding protein Rho, which is involved in the regulation of the actin cytoskeleton. The authors used Xenopus laevis oocytes as a model to study the toxin effect on Rho in more detail. Toxin B treatment of oocytes caused a decrease in subsequent ADP-ribosylation of cytoplasmic Rho by C3. This decrease was observed when toxin B was applied externally or after microinjection. Besides endogenous Rho, microinjected recombinant Rho-glutathione S-transferase **fusion** protein was affected. Impaired ADP-ribosylation of Rho was neither due to altered guanine nucleotide binding nor to complexation with the guanine nucleotide dissociation inhibitor, which is known to inactivate Rho and to prevent Rho modification by C3. Proteolytical degradation of Rho was excluded by immunoblot anal. In intact oocytes toxin B caused neither ADP-ribosylation nor phosphorylation of Rho. The data indicate that C. difficile toxin B acts on Rho proteins in Xenopus oocytes to inhibit ADP-ribosylation by C3. It is suggested that toxin B mediates its cytotoxic effect via functional inactivation of Rho.

L10 ANSWER 47 OF 53 EMBASE
on STN
94004871 EMBASE Document No.: 1994004871. Postoperative Clostridium difficile pseudomembranous colitis in idiopathic scoliosis: A brief clinical report. Osebold W.R.; Cohen A.N.; Gillum M.D.; Hurley J.H.; Locher N.J.. Specialty Orthopaedics, Sacred Heart Doctors Bldg., W. 105 8th Ave.,Spokane, WA 99220, United States. Spine 18/16 (2549-2552) 1993.
ISSN: 0362-2436. CODEN: SPINDD. Pub. Country: United States. Language: English. Summary Language: English.
AB The authors report two healthy young patients with progressive idiopathic scoliosis, both without allergies or histories of gastrointestinal disorders, who received perioperative preventive cephalosporin antibiotics, and developed explosive diarrhea postoperatively, confirmed as Clostridium difficile pseudomembranous colitis by stool toxin assay. Both patients had initially recovered uneventfully after posterior **fusion** and Cotrel-Dubousset instrumentation. Their youth, health, diagnosis, and lack of nosocomial factors made colitis unexpected. The two cases were sporadic, occurring 2 years apart over a 12-year observation period. Symptoms and signs of colitis for the two patients were markedly varied as to time of onset, order of appearance, and severity. Unexplained fever before onset of diarrhea led to renewed cephalosporin administration, potentially exacerbating the colitis. Initial symptoms and signs were nonspecific; appropriate treatment had to begin before diagnosis could be confirmed by stool toxin assay, which requires 2 days.

L10 ANSWER 48 OF 53 CAPLUS
1993:668374 Document No. 119:268374 Development of a molecular engineered vaccine for C. botulinum neurotoxins. LaPenotiere, H. F.; Clayton, M. A.; Brown, J. E.; Middlebrook, J. L. (Toxinol. Div., USAMRID, Fort Detrick, Frederick, MD, 21702, USA). Botulinum Tetanus Neurotoxins [Proc. Int. Conf.], Meeting Date 1992, 463-6. Editor(s): Dasgupta, Bibhuti R. Plenum: New York, N. Y. (English) 1993. CODEN: 59KIAW.
AB This report describes initial efforts to determine if botulinum neurotoxin type A Hc fragments will elicit a protective immune response. Using clones encoding part of the heavy chain, gene segment constructs were designed to produce a native Hc polypeptide or an Hc polypeptide fused to Escherichia coli maltose-binding protein. Problems were encountered with both systems. Specifically, the construct for the native protein appeared to be unstable, while the **fusion** product appeared to be packaged in inclusion bodies that formed insol. aggregates. Preliminary trials with animals indicated that vaccination with the **fusion** product conferred protection against native toxin challenge.

L10 ANSWER 49 OF 53 CAPLUS
1992:421259 Document No. 117:21259 Cloning of a Clostridium botulinum type B

toxin gene fragment encoding the N-terminus of the heavy chain. Jung, Hyun Ho; Rhee, Sang Dal; Yang, Kyu Hwan (Dep. Life Sci., Korea Adv. Inst. Sci. Technol., Taejon, 305-701, S. Korea). FEMS Microbiology Letters, 91(1), 69-72 (English) 1992. CODEN: FMLED7. ISSN: 0378-1097.

AB Two λgt11 clones of the toxin gene of C. botulinum type B were identified by the monoclonal antibody specific to the heavy chain of type B toxin. Neither of the expressed **fusion** proteins from the lysates of lysogenic Escherichia coli Y1089 showed any botulinal toxic activity. One of the clones hybridized to the oligonucleotide probe which was synthesized according to the amino acid sequence of N-terminus of heavy chain. The sequence anal. revealed that highly homologous regions in N-terminus of heavy chain exist among botulinum neurotoxins (type A, B) and tetanus toxin on the amino acid sequence level.

L10 ANSWER 50 OF 53 EMBASE
on STN

89099862 EMBASE Document No.: 1989099862. α-Latrotoxin and related toxins. Rosenthal L.; Meldolesi J.. Department of Pharmacology, CNR Center of Cytopharmacology and Scientific Institute S. Raffaele, Universita di Milano 20132 Milano, Italy. Pharmacology and Therapeutics 42/1 (115-134) 1989.
ISSN: 0163-7258. CODEN: PHTHDT. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB What have we learned of importance in terms of presynaptic physiology from the study of pure presynaptic stimulatory toxins, and what do we expect to learn in the near future? First, these toxins are likely to be addressed to the presynaptic compartment because of their specific high affinity interaction with protein receptors localized in the presynaptic membrane. So far, only one such receptor (the αLTx receptor) has been identified an purified, but the evidence about the others, although limited and indirect, appears unambiguous. Toxin receptors can therefore be added on to the (so far quite short) list of protein molecules specific for the presynaptic membrane, a region of the neuronal plasmalemma that is certainly highly specialized not only from the functional, but also from the molecular point of view. Second, toxins have been employed to investigate the Ca2+ dependence of evoked neurotransmitter release. In this respect, the pure presynaptic neurotoxins are not the only agents or treatments known to stimulate release even when Ca2+ is withdrawn from the incubation medium. Only with αLTx, however, has evoked release in a Ca2+-free incubation medium been shown to occur without changes of [Ca2+](i), i.e. without redistribution of Ca2+ from intraterminal stores to the cytoplasm. Finally, αLTx has proven to be an important tool in the kinetic and mechanistic studies of synaptic vesicle **fusion** and recycling, and a critical support to the now widely accepted vesicle hypothesis of quantal neurotransmitter release. In the face of these successes it is only fair to acknowledge that important clues to toxin action, with immediate correlation in terms of presynaptic physiology, remain mysterious. For example, our present knowledge of the functioning of the αLTx receptor is still very limited. Is the information leading to toxin action contained in the receptor, in the toxin, or in both these molecules together? Is the function of the receptor restricted to the binding and possibly transmembrane conveyance of the toxin message, or does it include other activities as well (for example, binding of endogenous ligands)? Are toxin receptors distributed over the entire presynaptic membrane, or localized at strategic sites, possibly at or near the **fusion** sites for synaptic vesicles? Finally, and even more importantly, what is the Ca2+-independent mechanism of exocytotic neurotransmitter release about? Is this process triggered by an action of the toxin entirely separate from the activation of ion fluxes (for example, by the generation of a second messenger?) Is this process responsible for the overcoming by αLTx of the presynaptic blockade by **Clostridium toxins**? These and many other open questions clearly demonstrate that the field of αLTx and congeners is destined to remain active and fertile in the near future. Answering at least part of these questions represents our challenge for the future, and

an important task in presynaptic physiology.

L10 ANSWER 51 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN
1989:109394 Document No. 110:109394 Cloning and characterization of overlapping DNA fragments of the toxin A gene of Clostridium difficile. Von Eichel-Streiber, Christoph; Suckau, Detlev; Wachter, Manfred; Hadding, Ulrich (Inst. Med. Mikrobiol., Johannes-Gutenberg-Univ., Mainz, 6500, Fed. Rep. Ger.). Journal of General Microbiology, 135(1), 55-64 (English) 1989. CODEN: JGMIAN. ISSN: 0022-1287.
AB C. difficile, a human pathogen, produces two very large protein toxins, A and B (250-600 kDa), which resist dissociation into subunits. To clone the toxin A gene, a genomic library of 3-8 kb chromosomal DNA fragments of C. difficile strain VPI 10463 established in pUC12 was screened with a rabbit polyclonal toxin A antiserum. Thirty-five clones were isolated which carried 2.5-7.0 kb inserts representing a 10 kb region of the C. difficile genome. All the inserts were oriented in the same direction, suggesting that toxin A gene expression was under control of the lac promoter of the pUC12 vector. Western blot expts. revealed the presence of low amts. of **fusion** proteins of variable size (30-170 kDa) in Escherichia coli strains harboring recombinant plasmids. As deduced from subcloning expts., the DNA sequences encoding toxin A comprised about 4 kb, corresponding to about 140 kDa of the 300-600 kDa protein. This was either due to incomplete cloning of the gene, or it might indicate a subunit composition of toxin A. No addnl. gene(s) with homol. to the cloned toxin A gene was detected.

L10 ANSWER 52 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN
1986:547882 Document No. 105:147882 Lysosomal involvement in cellular intoxication with Clostridium difficile toxin B. Florin, Inger; Thelestam, Monica (Dep. Bacteriol., Karolinska Inst., Stockholm, S-104 01, Swed.). Microbial Pathogenesis, 1(4), 373-85 (English) 1986. CODEN: MIPAEV. ISSN: 0882-4010.
AB After the internalization of C. difficile toxin B into human lung fibroblasts, cytopathogenic effects were studied. The development of the toxin-induced cytopathol. was reversibly inhibited at 18° and in the presence of 200 mM KCl or 1-20 mM benzyl alc. [100-51-6] under conditions when the **fusion** between endosomes and lysosomes is prevented. Fibroblasts treated with toxin at 37° but transferred to 18° within 10 min were also completely protected, whereas transfer to 18° later during the latency resulted in only partial protection. KCl was also protective upon addition after the toxin binding step. Inhibitors of lysosomal proteases, such as chymostatin [9076-44-2], leupeptin and antipain [37691-11-5], prevented the appearance of the cytopathogenic effect, when present during toxin exposure or added after the toxin binding step. Chinese hamster ovary cell mutants, defective in acidification of their endosomes, were resistant to toxin B, whereas wild-type cells were sensitive. The resistance were not overcome by applying a low extracellular pH. Apparently, exposure to a low pH compartment is necessary but not sufficient for entry of active toxin B to the cytosol. In addition to a low pH, a **fusion** of toxin-containing endosomes with lysosomes and a further processing of the toxin by lysosomal proteases is required for cellular intoxication.

L10 ANSWER 53 OF 53 CAPLUS COPYRIGHT 2005 ACS on STN
1983:3370 Document No. 98:3370 Four different monoclonal antibodies against type C1 toxin of Clostridium botulinum. Oguma, Keiji; Agui, Takashi; Syuto, Bunei; Kimura, Kazuyuki; Iida, Hiroo; Kubo, Shuichiro (Sch. Med., Hokkaido Univ., Sapporo, 060, Japan). Infection and Immunity, 38(1), 14-20 (English) 1982. CODEN: INFIBR. ISSN: 0019-9567.
AB Monoclonal antibodies against type C1 toxin produced by C. botulinum type C strain Stockholm (C-ST) were prepared by **fusion** of BALB/c myeloma cells P3X63-Ag8, with spleen cells from the mice immunized by C-ST toxoid. About 5% of single-cell colonies in wells were found to produce antibodies against the toxin as determined by ELISA. Four different hybridoma

cell lines, number 9, 12, 14, and 17, were established, cloned by limiting dilution, and i.p. injected into mice to obtain the ascites fluids containing high-titered antibodies. The reactions of these antibodies to type C1 and D toxins of strains C-ST, D-1873, and D-South African (D-SA) were observed by both neutralization and ELISA tests. Three monoclonal antibodies, number 9, 14, and 17, reacted with C-ST toxin, but only number 17 highly neutralized the toxin. These antibodies did not react with type D toxins. On the contrary, number 12 reacted with toxins of both C-ST and D-SA (but not of D-1873) and commonly neutralized these 2 toxins. This indicates that there is a common antigenic part between C-ST and D-SA toxin mols. which participates in the toxin-neutralizing reaction. The neutralization profiles of C-ST toxin by number 12 and 17 antibodies were different in a time-to-death test of mice. The mechanisms of neutralization by number 12 and 17 may be different.

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=> s (foster k?/au or chaddock j?/au or quinn c?/au or purkiss j?/au)
L11        4565 (FOSTER K?/AU OR CHADDOCK J?/AU OR QUINN C?/AU OR PURKISS J?/AU)


=> s l11 and clostridial neurotoxin
L12          32 L11 AND CLOSTRIDIAL NEUROTOXIN

=> dup remove l12
PROCESSING COMPLETED FOR L12
L13          17 DUP REMOVE L12 (15 DUPLICATES REMOVED)

=> d l13 1-17 cbib abs
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L13 ANSWER 1 OF 17 CAPLUS COPYRIGHT 2005 ACS on STN

2004:312287 Document No. 140:315072 Methods and compounds for the treatment of mucus hypersecretion by inhibiting mucus secretion using compounds having targeting and translocating modified light chain of **clostridial neurotoxin**. **Quinn, Conrad Padraig**; **Foster, Keith Alan**; **Chaddock, John** (Health Protection Agency, USA). U.S. Pat. Appl. Publ. US 2004071736 A1 20040415, 19 pp., Cont.-in-part of U.S. 6,632,440. (English). CODEN: USXXCO. APPLICATION: US 2003-633698 20030805. PRIORITY: GB 1998-18548 19980825; WO 1999-GB2806 19990825; US 2001-763669 20010529.

AB A method of treating mucus hypersecretion, the causative factor in chronic obstructive pulmonary disease (COPD), asthma and other clin. conditions involving COPD, comprises administering a compound that inhibits exocytosis in mucus secreting cells or neurons that control or direct mucus secretion. Also described is a compound, for use in the treatment of hypersecretion of mucus, which inhibits mucus secretion by inhibiting mucus secretion by mucus secreting cells, and/or inhibiting neurotransmitter release from neuronal cells controlling or directing mucus secretion. The compound comprises: (a) a light chain (L-chain) or L-chain fragment of a **clostridial neurotoxin**, which L-chain or L-chain fragment includes the active proteolytic enzyme domain of the L-chain; (b) a targeting domain that binds to a target cell selected from the group consisting of (i) a mucus secreting cell, and (ii) a neuronal cell controlling or directing mucus secretion; and (c) a translocating domain that translocates the L-chain or L-chain fragment into the target cell; with the proviso that the compound is not a botulinum toxin. Substance P, as the targeting domain, was conjugated to **clostridial neurotoxin** fragment LHN/A.

L13 ANSWER 2 OF 17 MEDLINE on STN DUPLICATE 1

2004530648. PubMed ID: 15500391. The analgesic potential of **clostridial neurotoxin** derivatives. **Foster Keith A.** (HPA Porton Down, Salisbury, Wiltshire, SP4 0JG, UK.. keith.foster@hpa.org.uk) . Expert opinion on investigational drugs, (2004 Nov) 13 (11) 1437-43. Journal code: 9434197. ISSN: 1744-7658. Pub. country: England: United Kingdom. Language: English.

AB Botulinum neurotoxins are the most potent acute lethal toxins known, and yet for the last two decades they, and in particular serotype A, have found increasing use in the clinical treatment of diseases or conditions involving neuromuscular or autonomic neuronal transmission. The neurotoxins work by inhibiting the release of acetylcholine from peripheral cholinergic nerve terminals. More recently, the effects on non-cholinergic pathways have been identified, and this has led to an increase in the diseases and syndromes for which botulinum neurotoxins have been found to have clinical utility. In particular, botulinum neurotoxins have been demonstrated to potentially benefit a range of chronic pain syndromes. With the description in the last decade of the biochemical basis of neurotoxin action and the tertiary structure of the toxin molecule, the possibility of designing novel agents utilising selected aspects of toxin function has arisen. This possibility has been pursued in the context of pain relief with the description of a novel hybrid protein derived from botulinum neurotoxin type A, LH(N)/A-ECL, able to selectively target nociceptive afferent neurons and inhibit the release of neurotransmitters involved in pain transmission. This novel derivative of botulinum neurotoxin type A demonstrates prolonged analgesic activity in vivo. This review will consider the evidence for the analgesic properties of the botulinum neurotoxins and their suitability as the basis for novel therapeutic proteins. The general concept of deriving novel therapeutic molecules from the neurotoxins will also be considered.

L13 ANSWER 3 OF 17 MEDLINE on STN DUPLICATE 2
2004134201. PubMed ID: 15027053. Retargeted clostridial endopeptidases: inhibition of nociceptive neurotransmitter release in vitro, and antinociceptive activity in in vivo models of pain. **Chaddock John A**; **Purkiss John R**; Alexander Frances C G; Doward Sarah; Fooks Sarah J; Friis Lorna M; Hall Yper H J; Kirby Elizabeth R; Leeds Nicola; Moulsdale Hilary J; Dickenson Anthony; Green G Mark; Rahman Wahida; Suzuki Rie; Duggan Michael J; **Quinn Conrad P**; Shone Clifford C; **Foster Keith A.** (Health Protection Agency, Porton Down, Salisbury, Wiltshire, United Kingdom.. john.chaddock@hpa.org.uk) . Movement disorders : official journal of the Movement Disorder Society, (2004 Mar) 19 Suppl 8 S42-7. Journal code: 8610688. ISSN: 0885-3185. Pub. country: United States. Language: English.

AB **Clostridial neurotoxins** potently and specifically inhibit neurotransmitter release in defined cell types. Previously reported data have demonstrated that the catalytically active LH(N) endopeptidase fragment of botulinum neurotoxin type A (termed LH(N)/A) can be retargeted to a range of cell types in vitro to lead to inhibition of secretion of a range of transmitters. Here, we report the synthesis of endopeptidase conjugates with in vitro selectivity for nociceptive afferents compared to spinal neurons. Chemical conjugates prepared between Erythrina cristagalli lectin and LH(N)/A are assessed in vitro and in in vivo models of pain. Chemical conjugates prepared between E. cristagalli lectin and either natively sourced LH(N)/A, or recombinant LH(N)/A purified from Escherichia coli are assessed, and equivalence of the recombinant material is demonstrated. The duration of action of inhibition of neurotransmitter release by the conjugate in vitro is also assessed and is comparable to that observed with Clostridium botulinum neurotoxin. Selectivity of targeting and therapeutic potential have been confirmed by in vivo electrophysiology studies. Furthermore, the analgesic properties of the conjugate have been assessed in in vivo models of pain and extended duration effects observed. These data provide proof of principle for the concept of retargeted clostridial endopeptidases as novel analgesics.

L13 ANSWER 4 OF 17 CAPLUS COPYRIGHT 2005 ACS on STN
2002:403839 Document No. 136:395977 Clostridial toxin derivatives able to modify peripheral sensory afferent functions. **Foster, Keith Alan** ; Duggan, Michael John; Shone, Clifford Charles (The Speywood Laboratory, Ltd., UK; Microbiological Research Authority). U.S. US 6395513 B1

20020528, 18 pp., Cont.-in-part of U.S. Ser. No. 945,037. (English). CODEN: USXXAM. APPLICATION: US 1999-447356 19991122. PRIORITY: GB 1995-8204 19950421; WO 1996-GB916 19960416; US 1998-945037 19980112.

AB The invention discloses an agent specific for peripheral sensory afferents. The agent may inhibit the transmission of signals between a primary sensory afferent and a projection neuron by controlling the release of at least one neurotransmitter or neuromodulator from the primary sensory afferent. The agent may be used in or as a pharmaceutical for the treatment of pain, particularly chronic pain. Agents of the invention include a modified **clostridial neurotoxin** fused to a targeting moiety. Preparation and biol. testing of a conjugate of NGF with the LHN fragment of botulinum neurotoxin A are included.

L13 ANSWER 5 OF 17 MEDLINE on STN DUPLICATE 3

2002470902. PubMed ID: 12105193. Inhibition of release of neurotransmitters from rat dorsal root ganglia by a novel conjugate of a Clostridium botulinum toxin A endopeptidase fragment and Erythrina cristagalli lectin. Duggan Michael J; **Quinn Conrad P**; **Chaddock John A**; **Purkiss John R**; Alexander Frances C G; Doward Sarah; Fooks Sarah J; Friis Lorna M; Hall Yper H J; Kirby Elizabeth R; Leeds Nicola; Moulsdale Hilary J; Dickenson Anthony; Green G Mark; Rahman Wahida; Suzuki Rie; Shone Clifford C; **Foster Keith A.** (Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 0JG, United Kingdom.) Journal of biological chemistry, (2002 Sep 20) 277 (38) 34846-52. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB **Clostridial neurotoxins** potently and specifically inhibit neurotransmitter release in defined cell types. Here we report that a catalytically active derivative (termed LH(N)/A) of the type A neurotoxin from Clostridium botulinum has been coupled to a lectin obtained from Erythrina cristagalli to form a novel conjugate. This conjugate exhibits an in vitro selectivity for nociceptive afferents compared with the anatomically adjacent spinal neurons, as assessed using in vitro primary neuronal culture systems to measure inhibition of release of neurotransmitters. Chemical conjugates prepared between E. cristagalli lectin and either natively sourced LH(N)/A or recombinant LH(N)/A purified from Escherichia coli are assessed, and equivalence of the recombinant material are demonstrated. Furthermore, the dependence of inhibition of neurotransmitter release on the cleavage of SNAP-25 is demonstrated through the use of an endopeptidase-deficient LH(N)/A conjugate variant. The duration of action of inhibition of neurotransmitter released by the conjugate in vitro is assessed and is comparable with that observed with Clostridium botulinum neurotoxin. Finally, in vivo electrophysiology shows that these in vitro actions have biological relevance in that sensory transmission from nociceptive afferents through the spinal cord is significantly attenuated. These data demonstrate that the potent endopeptidase activity of **clostridial neurotoxins** can be selectively retargeted to cells of interest and that inhibition of release of neurotransmitters from a neuronal population of therapeutic relevance to the treatment of pain can be achieved.

L13 ANSWER 6 OF 17 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2002:959295 The Genuine Article (R) Number: 613ZK. Botulinum and tetanus neurotoxins: structure, function and therapeutic utility. Turton K (Reprint); **Chaddock J A**; Acharya K R. Univ Bath, Dept Biol & Biochem, Claverton Down, Bath BA2 7AY, Avon, England (Reprint); Univ Bath, Dept Biol & Biochem, Bath BA2 7AY, Avon, England; Publ Hlth Lab Serv, Ctr Appl Microbiol & Res, Salisbury SP4 0JG, Wilts, England. TRENDS IN BIOCHEMICAL SCIENCES (NOV 2002) Vol. 27, No. 11, pp. 552-558. Publisher: ELSEVIER SCIENCE LONDON. 84 THEOBALDS RD, LONDON WC1X 8RR, ENGLAND. ISSN: 0968-0004. Pub. country: England. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The toxic products of the anaerobic bacteria Clostridium botulinum, Clostridium butyricum, Clostridium balrati and Clostridium tetani are the

causative agents of botulism and tetanus. The ability of botulinum neurotoxins to disrupt neurotransmission, often for prolonged periods, has been exploited for use in several medical applications and the toxins, as licensed pharmaceutical products, now represent the therapeutics of choice for the treatment for several neuromuscular conditions. Research into the structures and activities of botulinum and tetanus toxins has revealed features of these proteins that might be useful in the design of improved vaccines, effective inhibitors and novel biopharmaceuticals. Here, we discuss the relationships between structure, mechanism of action and therapeutic use.

L13 ANSWER 7 OF 17 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2002:519986 Document No.: PREV200200519986. Retargeted clostridial endopeptidases: Inhibition of nociceptive neurotransmitter release in vitro, and antinociceptive activity in in vivo models of pain. **Chaddock, J. A.** [Reprint author]; Duggan, M. J. [Reprint author]; Hall, Y. H. J. [Reprint author]; Kirby, E. R. [Reprint author]; Moulsdale, H. J. [Reprint author]; **Purkiss, J. R.** [Reprint author]; **Quinn, C. P.** [Reprint author]; Shone, C. C. [Reprint author]; Dickenson, A. H.; Cui, M.; Aoki, K. R.; **Foster, K. A.** [Reprint author]. Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 0JG, UK. Naunyn-Schmiedeberg's Archives of Pharmacology, (June, 2002) Vol. 365, No. Supplement 2, pp. R15. print. Meeting Info.: International Conference on Basic and Therapeutic Aspects of Botulinum and Tetanus Toxins. Hannover, Germany. June 08-12, 2002. CODEN: NSAPCC. ISSN: 0028-1298. Language: English.

L13 ANSWER 8 OF 17 CAPLUS COPYRIGHT 2005 ACS on STN

2001:228744 Document No. 134:247267 **Clostridial neurotoxin** targeted conjugates for inhibition of secretion from non-neuronal cells. **Foster, Keith Alan; Chaddock, John Andrew; Purkiss, John Robert; Quinn, Conrad Padraig** (Microbiological Research Authority, UK). PCT Int. Appl. WO 2001021213 A2 20010329, 63 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-GB3669 20000925. PRIORITY: GB 1999-22554 19990923.

AB A method of treatment of disease by inhibition of cellular secretory processes is provided. The method has particular application in the treatment of diseases dependent on the exocytotic activity of endocrine cells, exocrine cells, inflammatory cells, cells of the immune system, cells of the cardiovascular system, and bone cells. Agents and compns. therefor, as well as methods for manufacturing these agents and compns., are provided. In a preferred embodiment a **clostridial neurotoxin**, substantially devoid of holotoxin binding affinity for neuronal cells of the presynaptic muscular junction, is associated with a targeting moiety. The targeting moiety is selected such that the clostridial toxin conjugate so formed may be directed to a non-neuronal target cell to which the conjugate may bind. Following binding, a neurotoxin component of the conjugate, which is capable of inhibition of cellular secretion, passes into the cytosol of the target cell by cellular internalization mechanisms. Thereafter, inhibition of secretion from the target cell is effected.

L13 ANSWER 9 OF 17 CAPLUS COPYRIGHT 2005 ACS on STN

2002:238786 Document No. 137:196783 **Clostridial neurotoxins. Quinn, Conrad P.**; Minton, Nigel P. (Department of Molecular Microbiology, Centre for Applied Microbiology and Research, Salisbury, SP4 0JG, UK). Clostridia, 211-250. Editor(s): Bahl,

Hubert; Duerre, Peter. Wiley-VCH Verlag GmbH: Weinheim, Germany. ISBN: 3-527-30175-5 (English) 2001. CODEN: 69CKSR.

AB A review is given on the neurotoxins of Clostridium sp. The authors describe the genetic organization of the toxin genes, regulatory control of toxin gene expression, gene transfer in neurotoxinogenic clostridia, neurotoxin structure/function/mechanism of action, and therapeutic developments.

L13 ANSWER 10 OF 17 CAPLUS COPYRIGHT 2005 ACS on STN

2000:706999 Document No. 133:261538 Use of a lectin or lectin conjugate for modulation of C-fiber activity, and therapeutic use thereof. **Foster, Keith Alan; Chaddock, John Andrew; Quinn, Conrad Padraig** (Microbiological Research Authority, UK). PCT Int. Appl. WO 2000057897 A1 20001005, 62 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-GB1247 20000331. PRIORITY: GB 1999-7429 19990331.

AB The invention relates to the treatment of pain and to compds. that modulate C-fiber activity. In particular, the invention relates to the use of a lectin in the manufacture of a medicament for modulation of C-fiber neuron activity, and to lectin conjugates. The lectin conjugates comprise a lectin coupled to a peptide or protein, wherein the peptide or protein is substantially free of **Clostridial neurotoxin** enzyme activity. The invention also concerns methods for manufacturing the conjugates.

The compds. and compns. described have particular application in the treatment of diseases of which C-fiber activity is a component. Such diseases include pain, inflammation, psoriasis and other C-fiber related conditions.

L13 ANSWER 11 OF 17 CAPLUS COPYRIGHT 2005 ACS on STN

2000:144760 Document No. 132:175838 Compounds inhibiting exocytosis in mucus-secreting cells or neurotransmitter release from neurons that control or direct mucus secretion for treatment of mucus hypersecretion. **Quinn, Conrad Padraig; Foster, Keith Alan; Chaddock, John Andrew** (Microbiological Research Authority, UK). PCT Int. Appl. WO 2000010598 A2 20000302, 30 pp. DESIGNATED STATES: W: AU, CA, JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB2806 19990825. PRIORITY: GB 1998-18548 19980825.

AB A method of treating mucus hypersecretion, the causative factor in chronic obstructive pulmonary disease (COPD), asthma, and other clin. conditions involving COPD, comprises administering a compound that inhibits exocytosis in mucus secreting cells or neurons that control or direct mucus secretion. Also described is a compound, for use in the treatment of hypersecretion of mucus, which inhibits mucus secretion by inhibiting mucus secretion by mucus secreting cells, and/or inhibiting neurotransmitter release from neuronal cells controlling or directing mucus secretion.

L13 ANSWER 12 OF 17 MEDLINE on STN DUPLICATE 4

2000231793. PubMed ID: 10768948. Inhibition of vesicular secretion in both neuronal and nonneuronal cells by a retargeted endopeptidase derivative of Clostridium botulinum neurotoxin type A. **Chaddock J A; Purkiss J R**; Friis L M; Broadbridge J D; Duggan M J; Fooks S J; Shone C C; **Quinn C P; Foster K A.** (Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wiltshire SP4 0JG, United Kingdom.. john.chaddock@camr.org.uk) . Infection and immunity, (2000 May) 68 (5) 2587-93. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB **Clostridial neurotoxins** potently and specifically inhibit neurotransmitter release in defined cell types by a mechanism that involves cleavage of specific components of the vesicle docking/fusion complex, the SNARE complex. A derivative of the type A neurotoxin from Clostridium botulinum (termed LH(N)/A) that retains catalytic activity can be prepared by proteolysis. The LH(N)/A, however, lacks the putative native binding domain (H(C)) of the neurotoxin and is thus unable to bind to neurons and effect inhibition of neurotransmitter release. Here we report the chemical conjugation of LH(N)/A to an alternative cell-binding ligand, wheat germ agglutinin (WGA). When applied to a variety of cell lines, including those that are ordinarily resistant to the effects of neurotoxin, WGA-LH(N)/A conjugate potently inhibits secretory responses in those cells. Inhibition of release is demonstrated to be ligand mediated and dose dependent and to occur via a mechanism involving endopeptidase-dependent cleavage of the natural botulinum neurotoxin type A substrate. These data confirm that the function of the H(C) domain of C. botulinum neurotoxin type A is limited to binding to cell surface moieties. The data also demonstrate that the endopeptidase and translocation functions of the neurotoxin are effective in a range of cell types, including those of nonneuronal origin. These observations lead to the conclusion that a clostridial endopeptidase conjugate that can be used to investigate SNARE-mediated processes in a variety of cells has been successfully generated.

L13 ANSWER 13 OF 17 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

1999:910001 The Genuine Article (R) Number: 257VL. Sensitivity of embryonic rat dorsal root ganglia neurons to Clostridium botulinum neurotoxins. Welch M J; **Purkiss J R (Reprint)**; **Foster K A.** PUBL HLTH LAB SERV, CTR APPL MICROBIOL & RES, SALISBURY SP4 0JG, WILTS, ENGLAND (Reprint); PUBL HLTH LAB SERV, CTR APPL MICROBIOL & RES, SALISBURY SP4 0JG, WILTS, ENGLAND. TOXICON (FEB 2000) Vol. 38, No. 2, pp. 245-258. Publisher: PERGAMON-ELSEVIER SCIENCE LTD. THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND. ISSN: 0041-0101. Pub. country: ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Clostridium botulinum neurotoxins (BoNT) are zinc dependent endopeptidases which. once internalised into the neuronal cytosol, block neurotransmission :by proteolysis of membrane-associated proteins putatively involved in synaptic vesicle docking and fusion with the plasma membrane. Although many studies have used a variety of cellular systems to study the neurotoxins, most require relatively large amounts of toxin dr permeabilisation to internalise the neurotoxin. We present here a primary culture of embryonic rat dorsal root ganglia (DRG) neurons that exhibits calcium-dependent substance P secretion when depolarised with elevated extracellular potassium and is naturally BoNT sensitive. The DRG neurons showed a different IC50 for each of the toxins tested with a 1000 fold difference between the most and least potent neurotoxins (0.05, 0.3,30 and similar to 60 nM for A, C, F and B, respectively). BoNT/A cleavage of SNAP-25 was seen as early as 2 h, but substance P secretion was not significantly inhibited until 4 h intoxication and the effects of BoNT/A were observed for as long as 15 days. This primary neuronal culture system represents a new and sensitive cellular model for the ill vitro study of the botulinum neurotoxins. (C) 1999 Elsevier Science Ltd. All rights reserved.

L13 ANSWER 14 OF 17 MEDLINE on STN DUPLICATE 5

2000464599. PubMed ID: 11019785. A conjugate composed of nerve growth factor coupled to a non-toxic derivative of Clostridium botulinum neurotoxin type A can inhibit neurotransmitter release in vitro. **Chaddock J A**; **Purkiss J R**; Duggan M J; **Quinn C P** ; Shone C C; **Foster K A.** (Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, UK.) Growth factors (Chur, Switzerland), (2000) 18 (2) 147-55. Journal code: 9000468. ISSN: 0897-7194. Pub. country: Switzerland. Language: English.

AB Nerve growth factor (NGF) receptor binding, internalisation and transportation of NGF has been identified as a potential route of delivery for other molecules. A derivative of Clostridium botulinum neurotoxin type A (LHN) that retains catalytic activity but has significantly reduced cell-binding capability has been prepared and chemically coupled to NGF. Intact **clostridial neurotoxins** potently inhibit neurotransmitter release at the neuromuscular junction by proteolysis of specific components of the vesicle docking/fusion complex. Here we report that the NGF-LHN/A conjugate, when applied to PC12 cells, significantly inhibited neurotransmitter release and cleaved the type A toxin substrate. This work represents the successful use of NGF as a targeting moiety for the delivery of a neurotoxin fragment.

L13 ANSWER 15 OF 17 CAPLUS COPYRIGHT 2005 ACS on STN
1999:249106 Document No. 130:276767 Conjugates of galactose-binding lectins and **clostridial neurotoxins** as analgesics. Duggan, Michael John; **Chaddock, John Andrew** (The Speywood Laboratory Limited, UK; Microbiological Research Authority). PCT Int. Appl. WO 9917806 A1 19990415, 50 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-GB3001 19981007. PRIORITY: GB 1997-21189 19971008.

AB A class of novel agents that are able to modify nociceptive afferent function is provided. The agents may inhibit the release of neurotransmitters from discrete populations of neurons and thereby reduce or preferably prevent the transmission of afferent pain signals from peripheral to central pain fibers. They comprise a galactose-binding lectin linked to a derivative of a **clostridial neurotoxin.** The derivative of the **clostridial neurotoxin** comprises the L-chain, or a fragment thereof, which includes the active proteolytic enzyme domain of the light (L) chain, linked to a mol. or domain with membrane-translocating activity. The agents may be used in or as pharmaceuticals for the treatment of pain, particularly chronic pain.

L13 ANSWER 16 OF 17 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
97:386716 The Genuine Article (R) Number: WY820. Botulinum neurotoxin B inhibits insulin-stimulated glucose uptake into 3T3-L1 adipocytes and cleaves cellubrevin unlike type A toxin which failed to proteolyze the SNAP-23 present. Chen F S; Foran P; Shone C C; **Foster K A**; Melling J; Dolly J O (Reprint). UNIV LONDON IMPERIAL COLL SCI TECHNOL & MED, DEPT BIOCHEM, LONDON SW7 2AY, ENGLAND (Reprint); UNIV LONDON IMPERIAL COLL SCI TECHNOL & MED, DEPT BIOCHEM, LONDON SW7 2AY, ENGLAND; PUBL HLTH LAB SERV, CTR APPL MICROBIOL & RES, SALISBURY SP4 0JG, WILTS, ENGLAND. BIOCHEMISTRY (13 MAY 1997) Vol. 36, No. 19, pp. 5719-5728. Publisher: AMER CHEMICAL SOC. 1155 16TH ST, NW, WASHINGTON, DC 20036. ISSN: 0006-2960. Pub. country: ENGLAND. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Types A, B, and C1 botulinum neurotoxin (BoNT), a group of selective Zn2+-dependent endoproteases, have been instrumental in demonstrating that their respective substrates [synaptosomal-associated protein with M-r = 25 kDa (SNAP-25), synaptobrevin (Sbr), and syntaxin] are essential for regulated exocytosis from nerve terminals and neuroendocrine cells. The colocalization of Sbr, or its homologue cellubrevin (Cbr), in the majority of the glucose transporter-isotype 4 (GLUT4)-containing vesicles from adipocytes implicates their involvement in insulin-stimulated glucose uptake, which results in part from enhanced fusion of these vesicles with the plasmalemma. In this study, exposure of cultured 3T3-L1 adipocytes to BoNT/B in a low-ionic strength medium was found to block insulin-evoked glucose uptake by up to 64%. BoNT/B was shown by immunoblotting to cause

extensive proteolysis of Cbr and Sbr resulting in a significant blockade of the insulin-stimulated translocation of GLUT4 to the plasmalemma. This establishes that these two toxin substrates contribute to the insulin-regulated fusion of GLUT4-containing vesicles with the plasmalemma, at least in this differentiated 3T3-L1 clone. Although SNAP-25 was not detectable in the differentiated adipocytes, its functional homologue SNAP-23 is abundant and largely confined to the plasmalemma. SNAP-23 proved to be resistant to cleavage by BoNT/A. Consistent with these results, type A did not block insulin-induced glucose uptake, precluding a demonstration of its likely importance in this process.

L13 ANSWER 17 OF 17 CAPLUS COPYRIGHT 2005 ACS on STN
1999:442885 Document No. 131:266371 Novel therapeutic agents. **Foster, Keith A.** (CAMR, Salisbury, SP4 0JG, UK). Biomedical Aspects of Clostridial Neurotoxins, Conference, Oxford, July 8-11, 1996, Meeting Date 1996, 89-91. Editor(s): Tranter, Howard S. Centre for Applied Microbiology and Research: Salisbury, UK. (English) 1997. CODEN: 67WQAB.
AB A review with 27 refs. It is possible to allocate discrete biol. functions to individual domains of the **clostridial neurotoxin** polypeptide structure. These sep. components of toxin function offer opportunities for developing novel therapeutics.

```
=> s l3 and IL8
L14             0 L3 AND IL8

=> s l12 and IL8
L15             0 L12 AND IL8

=> d his
     (FILE 'HOME' ENTERED AT 10:12:10 ON 26 JAN 2005)

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     26 JAN 2005
L1               0 S CLOSTRIDIUM TOXIN COMPLEX
L2               0 S CLOSTRIDIUM TOXIN FUSION PROTEIN
L3            1905 S CLOSTRIDIUM TOXIN
L4              15 S L3 AND TARGETING
L5              14 DUP REMOVE L4 (1 DUPLICATE REMOVED)
L6               0 S L3 AND "NON-NEURONAL CELL"
L7              26 S L3 AND CONJUGATE
L8              25 DUP REMOVE L7 (1 DUPLICATE REMOVED)
L9              58 S L3 AND FUSION
L10             53 DUP REMOVE L9 (5 DUPLICATES REMOVED)
L11           4565 S (FOSTER K?/AU OR CHADDOCK J?/AU OR QUINN C?/AU OR PURKISS J?/
L12             32 S L11 AND CLOSTRIDIAL NEUROTOXIN
L13             17 DUP REMOVE L12 (15 DUPLICATES REMOVED)
L14              0 S L3 AND IL8
L15              0 S L12 AND IL8

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L16             7 L11 AND IL-8

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PROCESSING COMPLETED FOR L16
L17             3 DUP REMOVE L16 (4 DUPLICATES REMOVED)

=> d l17 1-3 cbib abs

L17 ANSWER 1 OF 3 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on
     STN
2001:146745 The Genuine Article (R) Number: 398ZM. Microparticulate uptake
     mechanisms of in-vitro cell culture models of the respiratory epithelium.
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Foster K A; Yazdanian M; Audus K L (Reprint). 236B Simons, 2095 Constant Ave, Lawrence, KS 66047 USA (Reprint); Univ Kansas, Dept Pharmaceut Chem, Lawrence, KS 66047 USA; Boehringer Ingelheim Pharmaceut Inc, Ridgefield, CT 06877 USA; GD Searle & Co, Pharmaceut Dev, Skokie, IL 60070 USA. JOURNAL OF PHARMACY AND PHARMACOLOGY (JAN 2001) Vol. 53, No. 1, pp. 57-66. Publisher: ROYAL PHARMACEUTICAL SOC GREAT BRITAIN. 1 LAMBETH HIGH ST, LONDON SE1 7JN, ENGLAND. ISSN: 0022-3573. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The objective of this study was to examine the uptake mechanisms of fluorescent polystyrene microspheres of various diameters and surface chemistry by two human cell lines derived from the respiratory epithelium, A549 and Calu-3.

Briefly, A549 and Calu-3 cells were grown to confluence in 12-well cluster plates and the uptake of fluorescent microspheres by the cells was determined at various time points. The amount of microspheres internalized by the cells was determined by correcting for non-specific binding to the cell surface. The data showed that A549 cells appeared to have more phagocytic activity than Calu-3 cells. Albumin-coated microspheres as large as 3 mum diameter can be internalized by A549 cells. The amount of internalization by A549 cells observed for 0.5-mum diameter albumin-coated microspheres was approximately 10-times greater than that observed for 1-mum diameter spheres and approximately 100-times greater than values observed for 2- and 3-mum diameter beads. Transmission electron micrographs confirmed that the microspheres were internalized by the cells. Uptake experiments conducted with Calu-3 cells indicated that albumin-coated microspheres were neither bound nor internalized by the cells. The effect of microsphere surface chemistry on the uptake mechanism indicated that amidine microspheres were internalized more rapidly and to a greater extent by both A549 and Calu-3 cells than carboxylate microspheres and non-coated microspheres. This phenomenon is thought to be attributed to masking of the negative polystyrene core by the positive amidine functional group; this effect was less marked for the carboxylate microspheres.

These results suggest that A549 and Calu-3 cells can internalize microspheres and that size and effective charge played an important role in the uptake process.

L17 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 1

97190134. PubMed ID: 9038289. Presence of high levels of leukocyte-associated interleukin-8 upon cell activation and in patients with sepsis syndrome. Marie C; Fitting C; Cheval C; Losser M R; Carlet J; Payen D; **Foster K**; Cavaillon J M. (Unite d'Immuno-Allergie, Institut Pasteur, Paris, France.) Infection and immunity, (1997 Mar) 65 (3) 865-71. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB In inflammatory and infectious diseases, the presence of circulating cytokines in plasma strongly suggests, following their exacerbated production, that saturation of specific binding sites has occurred or that an equilibrium between receptor-bound and free cytokines has been reached. In this report, we demonstrate that in addition to circulating interleukin-8 (**IL-8**), high levels of cell-associated **IL-8** were detected in blood samples from patients with sepsis syndrome. The following analysis will reveal that in addition to erythrocytes, which have been dubbed a "sink" for **IL-8**, peripheral blood mononuclear cells (PBMC) and polymorphonuclear cells (PMN) contributed to the detection of cell-associated **IL-8**. On a per cell basis, 2,000 to 7,000 times the amount of **IL-8** was found associated with PMN than with erythrocytes. In addition, circulating cells may well be the source of the leukocyte-associated form of **IL-8**. Similarly, in vitro experiments, such as whole-blood stimulation assays or the addition of exogenous **IL-8** in blood samples, demonstrated that a large proportion of the **IL-8** was associated with leukocytes. This suggests that the trapping of free cytokines onto the

cell surface and the internalization of the **IL-8** bound to its receptor, occurring both in vitro and in vivo, allows the detection of this cell-associated form. This analysis of cell-associated cytokines was extended to IL-1ra, another component of the inflammatory response, which, in contrast to **IL-8**, has been demonstrated to exist as an intracellular form. Indeed, cell-associated IL-1ra was also detected in septic patients. The measurement of cell-associated proinflammatory and anti-inflammatory cytokines in patients is clearly a more reliable reflection of their production than is the simple measurement in plasma and may provide useful indication to further understand the inflammatory process.

L17 ANSWER 3 OF 3 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
1997:50158 Document No.: PREV199799349361. High levels of leukocyte-associated **IL-8** upon LPS activation and in patients with sepsis.
Marie, C.; Pitton, C.; Fitting, C.; Cheval, C.; Losser, M.-R.; Carlet, J.; Payen, D.; **Foster, K.**; Cavaillon, J.-M.. Unite d'Immuno-Allergie, Inst. Pasteur, Paris, France. Journal of Endotoxin Research, (1996) Vol. 3, No. SUPPL. 1, pp. 61.
Meeting Info.: Fourth International Endotoxin Society Conference. Nagoya, Japan. October 22-25, 1996.
ISSN: 0968-0519. Language: English.

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=> s IL-8 fusion
L18           7 IL-8 FUSION

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PROCESSING COMPLETED FOR L18
L19           3 DUP REMOVE L18 (4 DUPLICATES REMOVED)

=> d l19 1-3 cbib abs
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L19 ANSWER 1 OF 3 MEDLINE on STN DUPLICATE 1
96429886. PubMed ID: 8833036. A fusion protein of IL-8 and a Fab antibody fragments binds to IL-8 receptors and induces neutrophil activation.
Holzer W; Petersen F; Strittmatter W; Matzku S; von Hoegen I.
(Pharmaceutical Research, Merck KGaA, Darmstadt, and University of Karlsruhe, Germany.) Cytokine, (1996 Mar) 8 (3) 214-21. Journal code: 9005353. ISSN: 1043-4666. Pub. country: United States. Language: English.

AB A fusion protein was generated by genetic engineering which combined a Fab fragment of a monoclonal antibody directed to the human epidermal growth factor receptor with the biologically active N-terminally truncated 2-72 amino acid form of the human chemokine IL-8. The Fab **IL-8 fusion** protein was expressed in E. coli and antibody binding and IL-8 activity were determined. Our data indicate that the N-terminus of IL-8 remains functional for receptor interaction. The fusion protein showed specific binding to IL-8 receptors, induced IL-8 mediated chemotactic activity, and the release of MPO activity. However, N-terminal fusion of IL-8 to the carboxyl terminus of the Fab fragment resulted in reduced binding to IL-8 receptors and consequently to reduced biologic activity of IL-8. The affinity of the antibody arm for EGF-R was improved when compared to a monovalent Fab. Fusion proteins as described herein may represent improved therapeutics for cancer therapy based on their potential to selectively increase and prolong cytokine concentration in the tumour. Since chemokines such as IL-8 recruit effector cells and stimulate effector cell function in situ, a lymphocyte-independent anti-tumour activity followed by tumour-specific immunity could be proposed.

L19 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN
1996:111454 Document No. 124:173036 Morphological alteration of canine neutrophils induced with recombinant canine interleukin-8. Matsumoto, Yasunobu; Matsumoto, Yoshitsugu; Onodera, Takashi; Yamagoe, Satoshi; Suzuki, Kazuo; Hirota, Yoshikazu (Faculty of Agriculture, University of

Tokyo, Tokyo, 113, Japan). Journal of Toxicologic Pathology, 8(3), 239-44 (English) 1995. CODEN: JTPAE7. ISSN: 0914-9198. Publisher: Japanese Society of Toxicologic Pathology.

AB Interleukin-8 (IL-8) is involved in the pathogenesis of a number of inflammatory diseases, such as rheumatoid arthritis, uveitis, pancreatitis, and ulcerative colitis. Mol. cloning of canine IL-8 was performed to establish a basis for its investigation in the canine immune system. Using an LPS-stimulated canine lymph node cDNA library as a template, the authors performed PCR amplification for the isolation of canine IL-8 cDNA. The 400-bp band amplified with the primers, was extracted from the gel and cloned into a pUC118 plasmid vector. The nucleotide sequences were determined Escherichia coli DH5α strain was used for the transformation and production of fusion protein containing glutathione-S-transferase (GST) and canine IL-8. Produced GST-canine **IL-8 fusion** protein was treated with Factor Xa and recombinant canine IL-8 was purified. Neutrophil shape change assay showed strong biol. activity of purified recombinant canine IL-8. To clarify the pathophysiol. role of IL-8, it is necessary to have an ELISA systems for the quantitation of IL-8 to increase the specificity and sensitivity for the evaluation of canine IL-8.

L19 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN

1995:766407 Document No. 123:167713 The conversion of human recombinant endothelial cell-derived **IL-8 fusion** proteins by thrombin. Zhou, Baohong; Ma, Dalong; Di, Chunhui; Song, Quansheng; Feng, Lan; Xiao, Bo.; Liu, Ji; Long, Zhenzhou (Dep. Immunol., Beijing Med. Univ., Beijing, 100083, Peop. Rep. China). Zhongguo Mianyixue Zazhi, 9(3), 147-51 (Chinese) 1993. CODEN: ZMZAEE. ISSN: 1000-484X. Publisher: Zhongguo Mianyixue Zazhi Bianjibu.

AB By using genetic engineering techniques, the authors expressed in E. coli fusion proteins which contained human endothelial cell-derived IL-8 (EDhIL-8), MS2 protein, and different length of β-galactosidase segments, named MS2-hIL-8, lac-hIL-8, lac-T-hIL-8 resp. The lact-T-hIL-8 has a synthesized thrombin recognition site. Because there is a natural thrombin recognition site within the EDhIL-8, thrombin can hydrolyze lac-hIL-8 and MS2-hIL-8 into natural hIL-8 exhibited biol. activity, but has no effect on lac-T-hIL-8 which contained two recognition sites for thrombin. These results here indicated that the recognition of thrombin dependents on not only the amino acid sequences of the substrates, but also the conformation formed by these amino acids.

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=> s IL-8
L20      48364 IL-8

=> s l20 and targeting
L21        359 L20 AND TARGETING

=> s l21 and fusion
L22          7 L21 AND FUSION

=> dup remove l22
PROCESSING COMPLETED FOR L22
L23           6 DUP REMOVE L22 (1 DUPLICATE REMOVED)

=> d l23 1-6 cbib abs
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L23 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN

2004:1037125 Document No. 142:33709 Methods and compositions related to high-titer pseudotyped retroviruses comprising a Jaagsiekte sheep retrovirus envelope glycoprotein and use for gene therapy. McCray, Paul; Fan, Hung; Sinn, Patrick (The University of Iowa Research Foundation, USA). PCT Int. Appl. WO 2004104032 A2 20041202, 72 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE,

GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-US14897 20040513. PRIORITY: US 2003-PV470326 20030514.

AB The present invention concerns methods and compns. related to pseudotyped viral vectors, particularly Jaagsiekte sheep retrovirus (JSRV)-feline immunodeficiency virus (FIV) vectors. Embodiments of the invention include pseudotyping expression cassettes that include nucleic acid elements for enhancing the titer of pseudotyped viral particles. Embodiments of the invention include methods and compns. related to making high titer pseudotyped retroviral vector compns. A heterologous envelope glycoprotein is typically incorporated into the virus during the budding or virus production process. Certain embodiments of the invention include pseudotyped retroviral vectors comprising a heterologous envelope glycoprotein derived from a Jaagsiekte sheep retrovirus (JSRV env), also known as ovine pulmonary adenocarcinoma virus. Pseudotyped viruses or viral particles may have a modified host range that is influenced by the properties of the heterologous envelope glycoprotein. Thus, embodiments of the invention include improved methods and compns. related to pseudotyped viruses suitable for ex vivo and in vivo methods including gene transfer and other therapeutic and exptl. methods. Efficient pseudotyping of feline immunodeficiency virus (FIV) results when the JSRV envelope expression plasmid retains the JSRV 3'-LTR and part of the JSRV 5'-LTR, including a major splice donor site. Examples of the invention diagram JSRV envelope protein expression cassettes and show expression of β-galactosidase in rabbit lungs after transduction with a pseudotyped JSRV-FIV vector.

L23 ANSWER 2 OF 6 MEDLINE on STN

2003208966. PubMed ID: 12729795. Von Willebrand factor targets **IL-8** to Weibel-Palade bodies in an endothelial cell line. Romani de Wit Thalia; de Leeuw Hubert P J C; Rondaij Mariska G; de Laaf Rozalia T M; Sellink Erica; Brinkman Herm-Jan; Voorberg Jan; van Mourik Jan A. (Department of Plasma Proteins and Blood Coagulation, CLB, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands.) Experimental cell research, (2003 May 15) 286 (1) 67-74. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB Vascular endothelial cells are able to store the chemotactic cytokine interleukin-8 (**IL-8**) in specialized storage vesicles, Weibel-Palade bodies, together with von Willebrand factor (VWF) and P-selectin. We investigated whether VWF plays a role in the sorting of **IL-8** into these organelles. We examined the effect of VWF expression on **IL-8 targeting** in an endothelial cell line (EC-RF24). This cell line has retained the typical phenotypic characteristics of primary endothelial cells but has lost the capacity to produce VWF in appreciable amounts. EC-RF24 cells were retrovirally transduced with a vector encoding a VWF-green fluorescent protein chimera (VWF-GFP). This approach enables direct visualization of the cellular distribution and secretory behavior of the VWF-GFP hybrid. Expression of VWF-GFP resulted in the generation of Weibel-Palade body-like organelles as shown by the colocalization of VWF-GFP and P-selectin. VWF-GFP expressing EC-RF24 cells also showed significant colocalization of VWF-GFP with **IL-8** in these storage vesicles. Live cell imaging revealed that the number of VWF-GFP-containing granules decreased upon cell stimulation. These observations indicate that VWF plays an active role in sequestering **IL-8** into Weibel-Palade bodies.

L23 ANSWER 3 OF 6 MEDLINE on STN DUPLICATE 1

2002299218. PubMed ID: 12040448. Bcr-abl-positive cells secrete angiogenic factors including matrix metalloproteinases and stimulate angiogenesis in vivo in Matrigel implants. Janowska-Wieczorek A; Majka M; Marquez-Curtis

L; Wertheim J A; Turner A R; Ratajczak M Z. (Department of Medicine, University of Alberta, Edmonton, Alberta, Canada.) Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K, (2002 Jun) 16 (6) 1160-6. Journal code: 8704895. ISSN: 0887-6924. Pub. country: England: United Kingdom. Language: English.

AB To further elucidate the role of angiogenesis in the pathogenesis of chronic myelogenous leukemia (CML) we evaluated the effects of the bcr-abl translocation on the secretion of the angiogenic factors VEGF, FGF-2, HGF, **IL-8** and matrix metalloproteinases (MMPs) as well as on the angiogenic potential in vivo of bcr-abl+ cells. First, we examined murine FL5.12 cells transfected with the bcr-abl constructs p185, p210 and p230 and found that the transfected cells secreted as much as four-fold more VEGF (p185 > p210 >p230) than wild-type (wt) cells, as well as MMP-9 and MMP-2. When Matrigel fragments containing these bcr-abl+ cells were implanted subcutaneously in SCID or Balb-C mice they became significantly more vascularized and hemoglobinized than implants containing normal or wt cells (p185 > p210 > p230). Similarly, we found that myeloblasts expanded from bone marrow (BM) CD34+ cells derived from Philadelphia-positive CML patients secreted up to 10 times more VEGF, FGF-2, HGF and **IL-8** compared to myeloblasts derived from normal donors' BM CD34+ cells and that BM mononuclear cells (MNC) isolated from CML patients induced vascularization of Matrigel implants in mice. Moreover, we found that peripheral blood MNC expressed MMP-2 and membrane-type (MT)1-MMP in about 50% of CML patients studied, and MMP-9 in all of them. Furthermore, VEGF stimulated the secretion of MMP-9 in these primary CML cells. We conclude that stimulation of angiogenesis by angiogenic factors, including MMPs, could play an important role in the pathogenesis of CML, suggesting that therapies **targeting** the newly formed endothelium could be developed for CML.

L23 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
2001:338579 Document No. 134:365705 Antibody diversity generation. Karrer, Erik; Bass, Steven H.; Whalen, Robert; Patten, Phillip A. (Maxygen, Inc., USA). PCT Int. Appl. WO 2001032712 A2 20010510, 109 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US30247 20001101. PRIORITY: US 1999-PV163370 19991103; US 2000-PV176002 20000112.

AB Methods for improving antibodies by a variety of DNA diversification and selection procedures are provided. Improvements include increases in affinity, alterations in specificity and effector function, as well as reduced antigenicity, e.g. humanization. Libraries of recombinant antibody sequences are provided, as are cells expressing members of such libraries. Novel phage display vectors are provided. Methods for the coevolution of an antibody and its cognate antigen are provided. Coevolution is used to evolve HIV envelope proteins with increased antigenicity and broadly neutralizing antibodies that interact therewith. Methods of improving antibodies for use in the detection of biol. warfare agents are provided.

L23 ANSWER 5 OF 6 MEDLINE on STN
1999008515. PubMed ID: 9794384. Adenovirus-mediated expression of a dominant negative mutant of p65/RelA inhibits proinflammatory gene expression in endothelial cells without sensitizing to apoptosis. Soares M P; Muniappan A; Kaczmarek E; Koziak K; Wrighton C J; Steinhauslin F; Ferran C; Winkler H; Bach F H; Anrather J. (Immunobiology Research Center, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115, USA.) Journal of immunology (Baltimore, Md. : 1950), (1998 Nov 1) 161 (9) 4572-82. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB We hypothesized that blocking the induction of proinflammatory genes associated with endothelial cell (EC) activation, by inhibiting the transcription factor nuclear factor kappaB (NF-kappaB), would prolong survival of vascularized xenografts. Our previous studies have shown that inhibition of NF-kappaB by adenovirus-mediated overexpression of I kappaB alpha suppresses the induction of proinflammatory genes in EC. However, I kappaB alpha sensitizes EC to TNF-alpha-mediated apoptosis, presumably by suppressing the induction of the NF-kappaB-dependent anti-apoptotic genes A20, A1, manganese superoxide dismutase (MnSOD), and cellular inhibitor of apoptosis 2. We report here that adenovirus mediated expression of a dominant negative C-terminal truncation mutant of p65/RelA (p65RHD) inhibits the induction of proinflammatory genes, such as E-selectin, ICAM-1, VCAM-1, **IL-8**, and inducible nitric oxide synthase, in EC as efficiently as does I kappaB alpha. However, contrary to I kappaB alpha, p65RHD does not sensitize EC to TNF-alpha-mediated apoptosis although both inhibitors suppressed the induction of the anti-apoptotic genes A20, A1, and MnSOD equally well. We present evidence that this difference in sensitization of EC to apoptosis is due to the ability of p65RHD, but not I kappaB alpha, to inhibit the constitutive expression of c-myc, a gene involved in the regulation of TNF-alpha-mediated apoptosis. These data demonstrate that it is possible to block the expression of proinflammatory genes during EC activation by **targeting** NF-kappaB, without sensitizing EC to apoptosis and establishes the role of c-myc in controlling induction of apoptosis during EC activation. Finally, these data provide the basis for a potential approach to suppress EC activation in vivo in transgenic pigs to be used as donors for xenotransplantation.

L23 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
1997:592254 Document No. 127:246717 Chemokine, as a target to intervene inflammation. Wada, Takashi; Yokoyama, Hitoshi; Kobayashi, Ken-ichi; Mukaida, Naofumi; Matsushima, Kouji (Department of First Internal Medicine, Kanazawa University, Kanazawa, 920, Japan). Ensho, 17(4), 335-343 (Japanese) 1997. CODEN: ENSHEE. ISSN: 0389-4290. Publisher: Nippon Ensho Gakkai Jimukyoku.

AB A review with 22 refs. Glomerular infiltration by neutrophils is a hallmark of acute glomerulonephritis. The pathophysiol. role of interleukin-8 (**IL-8**), a potent neutrophil chemotactic cytokine (chemokine), was explored in an animal model of acute immune complex-mediated glomerulonephritis by administering a neutralizing antibody against **IL-8**. Repeated injection of bovine serum albumin (BSA) into rabbits caused the deposition of immune complexes consisting of BSA and rabbit IgG in glomeruli. Histol. analyses revealed a small but significant number of neutrophils in glomeruli and the **fusion** of epithelial cell foot processes. Concomitantly, urinary levels of protein and albumin increased markedly (3.20 and 1.39 mg/h, resp.) compared with those of untreated animals (0.77 and 0.01 mg/h, resp.). Anti-**IL-8** antibody treatment decreased the number of neutrophils in glomeruli by 40% and dramatically prevented the **fusion** of epithelial cell foot process. Furthermore, treatment with anti-**IL-8** antibody completely normalized the urinary levels of protein and albumin (0.89 and 0.02 mg/h, resp.). These results indicated that **IL-8** participated in the impairment of renal functions in exptl. acute immune complex-mediated glomerulonephritis through activating as well as recruiting neutrophils. Here, we will overview the roles of chemokine in human diseases and discuss our therapeutic approaches to intervene inflammation **targeting** chemokine.

=> s l11 and WGA conjguate
L24 0 L11 AND WGA CONJGUATE

=> s l11 and WGA conjugate
L25 0 L11 AND WGA CONJUGATE

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=> s l11 and "WGA"
L26           6 L11 AND "WGA"

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PROCESSING COMPLETED FOR L26
L27           2 DUP REMOVE L26 (4 DUPLICATES REMOVED)

=> d l27 1-2 cbib abs

L27  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:144760   Document No. 132:175838  Compounds inhibiting exocytosis in
     mucus-secreting cells or neurotransmitter release from neurons that
     control or direct mucus secretion for treatment of mucus hypersecretion.
     Quinn, Conrad Padraig; Foster, Keith Alan;
     Chaddock, John Andrew (Microbiological Research Authority, UK).
     PCT Int. Appl. WO 2000010598 A2 20000302, 30 pp.  DESIGNATED STATES: W:
     AU, CA, JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT,
     LU, MC, NL, PT, SE.  (English).  CODEN: PIXXD2.  APPLICATION: WO
     1999-GB2806 19990825.  PRIORITY: GB 1998-18548 19980825.
AB   A method of treating mucus hypersecretion, the causative factor in chronic
     obstructive pulmonary disease (COPD), asthma, and other clin. conditions
     involving COPD, comprises administering a compound that inhibits exocytosis
     in mucus secreting cells or neurons that control or direct mucus
     secretion.  Also described is a compound, for use in the treatment of
     hypersecretion of mucus, which inhibits mucus secretion by inhibiting
     mucus secretion by mucus secreting cells, and/or inhibiting
     neurotransmitter release from neuronal cells controlling or directing
     mucus secretion.

L27  ANSWER 2 OF 2     MEDLINE on STN                          DUPLICATE 1
2000231793.    PubMed ID: 10768948.   Inhibition of vesicular secretion in both
     neuronal and nonneuronal cells by a retargeted endopeptidase derivative of
     Clostridium botulinum neurotoxin type A. Chaddock J A;
     Purkiss J R; Friis L M; Broadbridge J D; Duggan M J; Fooks S J;
     Shone C C; Quinn C P; Foster K A. (Centre for Applied
     Microbiology & Research, Porton Down, Salisbury, Wiltshire SP4 0JG, United
     Kingdom.. john.chaddock@camr.org.uk) . Infection and immunity, (2000 May)
     68 (5) 2587-93.  Journal code: 0246127. ISSN: 0019-9567. Pub. country:
     United States. Language: English.
AB   Clostridial neurotoxins potently and specifically inhibit neurotransmitter
     release in defined cell types by a mechanism that involves cleavage of
     specific components of the vesicle docking/fusion complex, the SNARE
     complex.  A derivative of the type A neurotoxin from Clostridium botulinum
     (termed LH(N)/A) that retains catalytic activity can be prepared by
     proteolysis.  The LH(N)/A, however, lacks the putative native binding
     domain (H(C)) of the neurotoxin and is thus unable to bind to neurons and
     effect inhibition of neurotransmitter release.  Here we report the
     chemical conjugation of LH(N)/A to an alternative cell-binding ligand,
     wheat germ agglutinin (WGA).  When applied to a variety of cell
     lines, including those that are ordinarily resistant to the effects of
     neurotoxin, WGA-LH(N)/A conjugate potently inhibits secretory
     responses in those cells.  Inhibition of release is demonstrated to be
     ligand mediated and dose dependent and to occur via a mechanism involving
     endopeptidase-dependent cleavage of the natural botulinum neurotoxin type
     A substrate.  These data confirm that the function of the H(C) domain of
     C. botulinum neurotoxin type A is limited to binding to cell surface
     moieties.  The data also demonstrate that the endopeptidase and
     translocation functions of the neurotoxin are effective in a range of cell
     types, including those of nonneuronal origin.  These observations lead to
     the conclusion that a clostridial endopeptidase conjugate that can be used
     to investigate SNARE-mediated processes in a variety of cells has been
     successfully generated.
```

```
=> s l11 and wheat germ aglutinin
L28           0 L11 AND WHEAT GERM AGLUTININ

=> s l3 and "HIT-T15 cells"
L29           0 L3 AND "HIT-T15 CELLS"

=> s "WGA-LHN A"
L30           3 "WGA-LHN A"

=> dup remove l30
PROCESSING COMPLETED FOR L30
L31            1 DUP REMOVE L30 (2 DUPLICATES REMOVED)

=> d l31 cbib abs

L31  ANSWER 1 OF 1  BIOSIS  COPYRIGHT (c) 2005 The Thomson Corporation.  on STN
     DUPLICATE 1
2000:224270  Document No.: PREV200000224270. Inhibition of vesicular secretion
     in both neuronal and nonneuronal cells by a retargeted endopeptidase
     derivative of Clostridium botulinum neurotoxin type A. Chaddock, John A.
     [Reprint author]; Purkiss, John R.; Friis, Lorna M.; Broadbridge, Janice
     D.; Duggan, Michael J.; Fooks, Sarah J.; Shone, Clifford C.; Quinn, Conrad
     P.; Foster, Keith A.. Centre for Applied Microbiology and Research, Porton
     Down, Salisbury, Wiltshire, SP4 0JG, UK. Infection and Immunity, (May,
     2000) Vol. 68, No. 5, pp. 2587-2593. print.
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     (termed LHN/A) that retains catalytic activity can be prepared by
     proteolysis.  The LHN/A, however, lacks the putative native binding domain
     (HC) of the neurotoxin and is thus unable to bind to neurons and effect
     inhibition of neurotransmitter release.  Here we report the chemical
     conjugation of LHN/A to an alternative cell-binding ligand, wheat germ
     agglutinin (WGA).  When applied to a variety of cell lines, including
     those that are ordinarily resistant to the effects of neurotoxin,
     WGA-LHN/A conjugate potently inhibits
     secretory responses in those cells.  Inhibition of release is demonstrated
     to be ligand mediated and dose dependent and to occur via a mechanism
     involving endopeptidase-dependent cleavage of the natural botulinum
     neurotoxin type A substrate.  These data confirm that the function of the
     HC domain of C. botulinum neurotoxin type A is limited to binding to cell
     surface moieties.  The data also demonstrate that the endopeptidase and
     translocation functions of the neurotoxin are effective in a range of cell
     types, including those of nonneuronal origin.  These observations lead to
     the conclusion that a clostridial endopeptidase conjugate that can be used
     to investigate SNARE-mediated processes in a variety of cells has been
     successfully generated.

=>

---Logging off of STN---


=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
```

FULL ESTIMATED COST	429.35	429.56
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-44.53	-44.53

STN INTERNATIONAL LOGOFF AT 10:26:24 ON 26 JAN 2005